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Tangled in transcription

The web of transcription factors regulating tomato type VI glandular trichome development and specialized metabolites

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Chapter 4

***Glandless*, a tomato HD-ZIP transcription factor, is important for gland formation of type VI trichomes**

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4.1 Abstract

Tomato (*Solanum lycopersicum*) is a model plant to study glandular trichomes development and their specialized metabolism, but only few of the transcription factors (TF) regulating these intertwined traits and their network have been characterized. Among them are members of the homeodomain leucine-zipper subfamily IV (HD-ZIP IV). Here, we study a tomato EMS-mutant line, *glandless*, presenting morphologically gland-less type VI glandular trichomes with a consequential reduction in volatile terpenes levels, and also with altered trichome densities. As verified via Virus Induced Gene Silencing (VIGS), the gene underlying this phenotype is SIHDZ38, first member of HD-ZIP subfamily I found to regulate the development and specialized metabolism of type VI glandular trichomes. Additionally, we show that the expression of an intricate network of known trichome-related regulatory TFs and biosynthetic enzymes is affected by the *glandless* mutation, differentially in leaf and stem trichomes. The characterization of SIHDZ38 via CRISPR-Cas9 knockouts revealed an additional role as type VI glandular trichomes are missing in these lines. Overall, our results contribute to the elucidation of the network of TFs controlling tomato trichomes.

4.2 Introduction

Among adaptations in vascular plants to interact with the environment, the hair-like structures called trichomes are found in approximately one third of the species with vastly diversified morphologies (Fahn, 2000; Payne, 1978). Glandular trichomes and their metabolites not only are central for the eco-physiology of plants to adapt to abiotic and biotic stresses or to interact with pollinators (Bickford, 2016; Wagner et al., 2004) but they have been exploited as high value ingredients in the food, cosmetic and pharmaceutical industry, and as natural pesticides in agriculture (Schillmiller et al., 2008). Tomato (*Solanum lycopersicum*) has become a model species widely studied to unravel the regulation of development and specialized metabolism of multicellular glandular trichomes in horticultural crops (Feng et al., 2021). In fact, cultivated tomato accessions and its wild relatives display different combinations and densities of eight type of trichomes, four non-glandular (type II, III, V and VIII) and four glandular (I, IV, VI, VII) (Glas et al., 2012; Luckwill, 1943). The latter are distinguished by their morphology, the number of cells and the chemical classes of compounds found in them (McDowell et al., 2011). Type VI trichomes are composed of a basal cell, a long stalk cell, a small intermediate cell and four glandular cells where mostly mono- and sesquiterpenes but also flavonoids and methylketones are produced (Akhtar et al., 2013; Bleeker, Diergaarde, et al., 2011; Fridman et al., 2005; Nakashima et al., 2016; Schillmiller et al., 2009) and deposited in the intercellular cavity in the middle of them (Bergau et al., 2015; Tissier et al., 2017b).

Up to now, only few tomato transcription factors (TF) have been characterized as regulators of type VI trichome development and metabolism, mostly belonging to the MYB (Myeloblastosys), bHLH (basic helix loop helix), ZFP (Zinc finger protein), GRAS (GAI, RGA, SCR) and HD-ZIP (Homeodomain leucine zipper) transcription factor (TF) families. The latter, only present in plants, is characterized by a homeobox domain (HD) and a leucine-zipper motif (ZIP) and comprises four classes (I-IV) that differ in functional domains (Y. Gao et al., 2015; Z. Zhang et al., 2014). To the subfamily IV belongs SIWO (WOOLLY), a master regulator of trichome type I, III, V, VI and VII differentiation (Vendemiatti et al., 2017; M. Wu et al., 2023; Yang, Li, Zhang, Luo, et al., 2011; Yang, Li, Zhang, Wang, et al., 2011). It has been recently suggested that trichome fate during development is controlled by SIWO via a dose-dependent mechanism. The SIWO protein concentration is regulated by self-activation of its gene and via negative feedback regulation by SIMTR1 and SIMTR2 (MULTICELLULAR TRICHOME REPRESSOR), previously characterized as trichome regulators with the name SICYCB2 and SICYCB3 (M. Wu et al., 2023). Low levels of SIWO activate *SILFS* (*LEAFLESS*) that promote gland formation during type VI and VII development, while high levels activate *SIMX1* (*MIXTA*) and *SIWOX3b* (*WUSCHEL-RELATED HOMEODOMAIN*) that form a complex and promote type I, III and V development also by repressing *SILFS* (M. Wu et al., 2023). *SILFS* expression is repressed also by SIGCR1 and 2 (GLAND CELL REPRESSOR), whose action is controlled by self-inhibition and SITOEB1 (TARGET OF EAT1 B) induced inhibition

(Chang et al., 2024). SIWO interacts independently with several of the other characterized trichome regulators. An additional role for SIMTR1 in regulating SIWO at the post-transcriptional level has been suggested because of their protein-protein interaction. (Wu et al., 2023; Yang et al., 2011). Specifically related to type VI glandular trichome development, it has been proposed that, under the regulation of Jasmonic Acid (JA) via SIJAZ2 (JASMONATE ZIM-DOMAIN) and SIJAZ4, SIWO interacts with SIMYC1 (MYELOCYTOMATOSIS). The resulting SIWO-SIMYC1 complex binds the promoters of *TPS* (TERPENE SYNTHASE) genes and activate their expression in mature type VI trichome gland cells (Hua et al., 2020; Hua et al., 2021). Additionally, SIWO binds to two C2H2 zinc-finger TFs known to regulate non-glandular trichomes, SIH (HAIR) and SISH (SPARSE HAIR) (Chang et al., 2018; Chun et al., 2021; Hua et al., 2022; R. Li et al., 2021; Zheng et al., 2022). It has been suggested that in this three-way interaction they cooperate in regulating the formation of multiple types of trichomes (Chang et al., 2018; Chun et al., 2021; Zheng et al., 2022). SILN (LANATA) is another recently characterized HD-ZIP IV transcription factor, whose natural mutant shows higher density of hairy trichomes (type I, III and V) and lower density of type VI and VII glandular trichomes (Xie et al., 2022). SILN interacts with H and binds to a L1-box in SIH promoter, positively regulating its expression. Moreover, similarly to SIWO, SILN binds a L1-box in the promoter of both *SIMTR1* and *SIMTR2*, activating their expression. SILN interacts with SIWO enhancing transcriptional activation of *SIMTR1* and *SIMTR2* and promoting trichome formation (Xie et al., 2022). To the HD-ZIP subfamily IV belongs also SICD2 (CUTIN DEFICIENT 2), a regulator of cuticle formation of epidermal cells that positively impacts type VI trichome density and volatile terpene levels (Nadakuduti et al., 2012). Lastly, the HD-ZIP IV TF SIHD8 (HOMEODOMAIN PROTEIN 8) is involved in trichome type I, III, V and VI initiation and morphogenesis by regulation of *SIHI* (*HAIRLESS*) and its homolog *SIHI-2*, coding respectively for two interacting subunits of the WAVE (WASP-family Verprolin-homologous protein) regulatory complex for actin filaments nucleation. SIHD8 function is required for the JA-induced trichome elongation by enhancing the expression of proteins determining the loosening of the cell wall and is regulated in this role by SIJAZ4 (Hua et al., 2021; Kang et al., 2010; Kang et al., 2016; Xie et al., 2020).

Although in the last decade many studies lead to the discovery of tomato genes involved in type VI glandular trichome development, a comprehensive regulatory model is far from being achieved. In this study we characterized *glandless*, a tomato mutant where type VI trichomes miss the glandular head cells and do not accumulate volatile terpenes. Additionally, we identified the mutated gene coding for a novel HD-ZIP TF, named *SIHDZ38*, the first of subfamily I ever linked to trichome regulation in tomato. With virus-induced silencing (VIGS) and CRISPR-Cas9 knockout we further confirmed that *SIHDZ38* is indispensable for the development of type VI trichomes and their glands, but also involved in controlling the densities of other trichome types. Altogether, these results provide new insights about the regulation of type VI glandular trichome development.

4.3 Materials and methods

Plant material and growing conditions

Tomato (*Solanum lycopersicum*) cv. Micro-Tom plants were germinated and grown in soil at 16/8 h and 23/18 °C day/night conditions in a greenhouse, supplemented when necessary with artificial light (150 mE m⁻²s⁻¹; Philips Master Green Power). The second and third couple of leaflets (counting from the leaf tip) of the fourth true leaf (counting from the plant top) of 4-week-old tomato plants were used for all the experiments, unless otherwise specified. *In-vitro* tissue and plant cultures were grown in a growth chamber (24°C, 70%RH, 16h light/8h dark) on ½ strength Murashige and Skoog (MS) medium (MS basal salts with vitamins 2.2 g/L, sucrose 5g/L, MES 1g/L, 0.8% agar, pH 5.8 with KOH) occasionally with the addition of the required supplements. Tomato breeding line Rijk Zwaan (RZ)-2 was mutagenized by submerging at room temperature, for 24h, 10.000 seeds in an aerated solution of 0.5% (w/v) ethyl methanesulfonate (EMS). The plants these seeds were grown in a greenhouse to produce M2 seeds. M2 seeds were harvested and bulked in one pool used to screen 8000 plants with a stereomicroscope to identify individuals exhibiting a type VI glandular trichome phenotype. By fine-mapping of the BC₁M₂ population obtained by backcrossing of the selected mutant line, the recessive

trait was localized on chromosome 9, specifically to the Solyc09g008810 gene. M6 seeds from the same selected mutants were used for all the phenotyping experiments.

Constructs generation

For the virus-induced silencing (VIGS) of the *SIHDZ38* gene (Solyc09g008810) (Hong et al., 2021), a 300 bp fragment of its cDNA sequence was selected via the Sol Genomics Network (SGN; <https://solgenomics.net>) VIGS tool (Fernandez-Pozo et al., 2015), synthesized with the addition at the 5' and 3' end of attL1 and attL2 gateway sites respectively (Gene Universal Inc., Newark DE, US, www.geneuniversal.com) and recombined via Gateway® LR clonase® II in pTRV2-2b vector (Valentine et al., 2004) producing pTRV2-2b-Solyc09g008810.

To generate *SIHDZ38*-knockout tomato lines via CRISPR-Cas9 genome editing, guide RNAs (gRNAs) were designed via CRISPR-P v2.0 (Lei et al., 2014; <http://crispr.hzau.edu.cn>) and CCTop (Stemmer et al., 2015; <https://cctop.cos.uni-heidelberg.de:8043/index.html>). The three sgRNAs were multiplexed in sequence, each one under the control of the *Arabidopsis thaliana* class III RNA polymerase U6 promoter, with the addition of attL5 and attL2 gateway sites respectively at the 5' and 3' end of the construct and cloned into pUC57 vector (Gene Universal Inc.). This was recombined, together with Cas9 entry vector pYPQ150 (Karimi et al., 2002) with the destination vector pK2GW7 in a multi-site reaction via Gateway® LR clonase® II (Invitrogen™; www.thermofisher.com) producing pK2GW7-CC-HDZ38 (Supplemental Figure 1). The sequences of all the generated constructs were verified by Sanger sequencing.

Tomato stable transformations

To generate transgenic tomato plants via *Agrobacterium tumefaciens*-mediated transformation (Cortina & Cullanez-Macia, 2004), pK2GW7-CC-HDZ38 was transformed by electroporation into *A. tumefaciens* strain GV3101 (pMP90) and plated on selective media. A single colony was selected and grown overnight at 28°C with 200rpm shaking in Luria Bertani (LB) low salt medium (Tryptone 10 g/L, Yeast extract 5g/L, NaCl 5g/L, Agar 10g/L) with antibiotics. From 10-days-old cotyledons of sterile seedlings of *S. lycopersicum* cv. Micro-Tom grown on ½ strength MS medium pH 5.6, squared explants were taken and placed overnight on co-culture medium (MS basal salts with vitamin B5 4,5g/L, sucrose 30g/L, MES 0,5g/L, Zeatin 2mg/L, Indole-3-acetic acid 0,1mg/L, 2,4 Dichlorophenoxyacetic acid 0,05mg/L, Acetosyringone 200 µM, 8% agar, pH 5.8 with KOH). 2,5ml of bacteria culture with OD₆₀₀ > 1,6 was inoculated in 25 ml LB medium, grown at 28°C with 200rpm shaking, until OD₆₀₀ = 0,8. After centrifugation at 3000rpm for 20min the bacteria culture was resuspended in 25ml Liquid medium (MS basal salts with vitamin B5 4,5g/L, sucrose 30g/L, MES 0,5g/L, Acetosyringone 200 µM, pH 5.8 with KOH) and left for > 1 hour at room temperature with 200 rpm shaking. The following day the explants were submerged for 5-10 min in the liquid *A. tumefaciens* culture, drained and transferred on new co-culture medium. After 48 hours, to kill *A. tumefaciens*, the explants were transferred on post-culture media (MS basal salts with vitamin B5 4,5g/L, sucrose 30g/L, MES 0,5g/L, Zeatin 2mg/L, Indole-3-acetic acid 0,1mg/L, Vancomycin 50mg/L, Cefotaxime 200mg/L, pH 5.8 with KOH). Two days later, the explants were transferred on Shoot-inducing medium (MS basal salts with vitamin B5 4,5g/L, D-glucose 10g/L, MES 0,5g/L, Zeatin 2mg/L, Indole-3-acetic acid 0,1mg/L, Carbenicillin 500mg/L, 8% agar, pH 5.8 with KOH) with 100 mg/L kanamycin as selective marker. Transformant shoots (T0) that progressively regenerated from the calli were moved to Rooting medium (MS basal salts with vitamin B5 4,5g/L, sucrose 15g/L, MES 0,5g/L, Cefotaxime 200mg/L, Indole-3-butyric acid 0.25 mg/L, 4% Gelrite, pH 5.8 with KOH). By PCR, the presence of the *nptII* gene in the genomic DNA was checked and plants containing it were transferred to soil, acclimated to greenhouse conditions, and grown to obtain seeds. T1 plants were grown and genotyped for the presence/absence of the *Cas9* gene in the genomic DNA via PCR and Qiaxcel capillary agarose system (QIAGEN, <https://www.qiagen.com>).

Sequences analyses and phylogenetic tree

To check the outcomes of the CRISPR-Cas9 mediated gene-editing, the amplified target gene was sequenced in isolated gDNA from all selected T1 plants with MinION and FLO-MIN112 flow cells (Oxford Nanopore Technologies, <https://nanoporetech.com/>). Basecalling was done with Guppy

(Oxford Nanopore Technologies) and the reads mapping via Minimap2 (H. Li, 2021). The final fastq files were then analyzed, to check the results of the gene editing, via CRISPResso2 (Clement et al., 2019) and then manually checked with IGV (Robinson et al., 2023). The 241 amino acids sequence of SIHDZ38 was obtained at SGN and used to search for homologous proteins in plant species by BLASTp against the National Center for Biotechnology Information (NCBI) reference protein database (refseq_protein). Putative homologs in several model plants species and crops were selected. The multiple sequence alignments were performed using the Toffee algorithm (Madeira et al., 2022) and visualized or edited with Jalview (Waterhouse et al., 2009). The phylogenetic tree was constructed in PhyML (Guindon et al., 2010) using the maximum-likelihood (ML) criterion with 500 bootstraps, and then optimized using the ggplot2 R package and Iroki (Moore et al., 2020).

Virus-induced gene silencing

For the virus-induced gene silencing (VIGS) assay (Liu et al., 2002) the tobacco rattle virus vector pTRV-2b-HDZ38 was transformed into *A. tumefaciens* GV3101 (pMP90) by electroporation. A single positive colony was grown overnight (28°C, 200rpm) in liquid LB medium and resuspended in infiltration buffer (MS basal salts without vitamins 4.44 g/L, Sucrose 20 g/L, 10mM MES, Acetosyringone 200 µM, pH 5.6 with NaOH) and left at room temperature for > 3 hours. An *A. tumefaciens* strain harboring a pTRV-2b vector targeting the tomato phytoene saturase gene SIPDS1 (Solyc03g123760) was used as control for the silencing. As negative control, an *A. tumefaciens* strain carrying a TRV2-2b targeting the eGFP gene was used. The three strains, respectively mixed in a 1:1 (v/v) ratio with another *A. tumefaciens* strain with the same OD₆₀₀ carrying the tobacco rattle virus vector pTRV1 (Liu et al., 2002) were infiltrated with a needleless syringe in cotyledons of 10-day-old tomato plants. Leaflets were collected from the plants four to six weeks after infiltration and used for microscopy, metabolomics and expression analyses.

RT quantitative PCR analyses

To isolate leaf RNA, the second pair of leaflets of the fourth true leaf counting from the bottom of 4-week-old plants were collected and immediately frozen in liquid nitrogen. To isolate trichome RNA, stems and petioles of 4-week-old whole plants were collected in 50ml tubes, frozen and shaken in liquid nitrogen with a vortex mixer. Total RNA was extracted using Trizol reagent (Invitrogen™) and isolated by treatment with TURBO DNase kit (Ambion™; www.thermofisher.com) to remove DNA. Quantity and quality of RNA was determined respectively with a NanoDrop (Thermo Scientific™; www.thermofisher.com) and by electrophoresis on 1% agarose gel. The synthesis of cDNA was performed with RevertAid H Minus Reverse Transcriptase (Thermo Scientific™) starting from 1-2 mg RNA. As a control for genomic DNA contaminations a sample with no RT enzyme was included. Quantitative PCRs (qPCR) were performed in 10ml reactions mixes containing 0.5 ml of cDNA (0.5-2 ng total RNA equivalent), 2ml HOT FIREPol EvaGreen qPCR Mix Plus (Solis Biodyne; https://solisbiodyne.com) and 4ml 300nM of each primer (Supplemental table 1) using a QuantStudio™ 3 Real-Time PCR System (Applied Biosystems™, www.thermofisher.com) and the following cycling program: 2min 50°C, 10min 95°C, 40 cycles of 15s 95°C and 1min 60°C. Primer pairs efficiency was calculated by analyzing a range of cDNA serial dilutions while a sample with no cDNA was included as control for primer dimers. Two technical replicates of three independent biological samples were analyzed. To calculate relative normalized expression levels, primers for the Actin housekeeping gene SIACT7 (Solyc03g078400) were used (Supplemental table 1).

Analyses of volatile terpenes

To analyze volatile terpenes in leaf trichomes, a leaflet of the second pair from the fourth true leaf counting from the bottom of 4-week-old plants was collected (n = 3-4), weighted and briefly washed (~ 5 s) with 750 µl or 1 ml cold n-hexane containing 0.5 ng/ml of either benzyl acetate or 1,2,3,4-Tetrahydronaphthalene (Sigma-Aldrich; www.sigmaaldrich.com) as internal standard. For stem trichomes, a segment of 7cm of the stem between the fourth and the fifth internode was sampled and briefly washed (~ 5 s) with 1 ml cold n-hexane containing 0.5 ng/ml internal standard. To quantify volatile terpenes in isolated type VI trichomes, 200 glandular head were manually collected under

stereomicroscope with a stretched glass Pasteur pipette that was rinsed every 20 trichomes into 150 ml cold n-hexane containing 0.5 ng/ml internal standard. To remove residues of water from all types of extracts, ~ 10 mg Na₂SO₄ (Sigma-Aldrich) was added to the sample. After vortexing for ~ 10 s, the extracts were centrifuged at 13.000 rpm for 5 min and the upper hexane layer was transferred in glass vials vented with N₂ to prevent oxidation, and stored at – 20 °C. An Agilent 7890A gas chromatograph coupled with an Agilent 7200 accurate-mass quadrupole time-of-flight (TOF) mass spectrometer (Agilent, www.agilent.com) were used for the gas chromatography mass spectrometry analysis. 1 ml of each sample was injected, heated to 275°C and separated on a HP-5ms capillary column (30 m × 250 mm; 0.25 mm thick; Agilent), using helium gas as carrier (7.0699 psi; 1 ml/min flow rate). After 5 min at 40°C the column oven temperature was increased first by 5°C/min until 140°C, then by 10°C/min up to 250°C and then kept at this temperature for 5 min. Ionization was done with electron-impact (EI) mode at 70 eV under vacuum. With a solvent delay of 4.1 min, detection of 30 to 350 m/z ions was achieved with 50 scans/second. MassHunter Qualitative Analysis software package (Agilent) was used for chromatogram peaks detection and deconvolution with 50 ppm accuracy. For identification and quantification, a mix of terpenes analytical standards, was injected in serial dilutions. Base-peak ion integration was used and the areas were normalized by internal standard, dilution volume and fresh-leaflet weight.

Microscopy

To observe and image trichome phenotype on leaves and stems, a stereomicroscope and an EVOS fl (Life Technologies, USA) inverted microscope were used. From 4-week-old plants, leaf disks of 0.6 cm diameter (n = 3) were taken on the second pair of leaflets of the fourth true leaf. Stem trichomes were observed on 1 cm long longitudinal sections of the main stem and the 4th-leaf petiole were taken right above the fourth internode. To study the morphology and density of trichomes with cryogenic scanning electron microscopy (Cryo-SEM), micrographs were taken at the Electron Microscopy Centre at Wageningen University (<http://www.wur.nl>). Leaf and stems explants were taken from 4-week-old plants and mounted on a brass sample holder using a thin layer of Tissue-Tek compound (EMS, Washington, PA, USA). Samples were frozen by plunging them into liquid nitrogen and subsequently placed in a cryo-preparation chamber (MED 020/VCT 100, Leica, Vienna, Austria). To sublimate any water vapor contamination (ice) from the surface, the samples were kept for 3 min at –93 °C at 2 × 10⁻⁶ mbar. Samples were then sputter coated with a 12 nm layer of tungsten, and transferred under vacuum to the field emission scanning electron microscope (Magellan 400, FEI, Eindhoven, the Netherlands) onto the sample stage at –120 °C. The images were taken with the secondary electron detector set at 2 kV, 13 pA, working distance 4mm. All images were recorded digitally with FEI maps software: the leaflet disks single images were taken at a scan rate of 100 s (full frame) with image size of 1536 × 1024 8 bit and then stitched together by the software; the stem sections were taken at a scan rate of 100 s (full frame) with image size of 1536 × 1024 8 bit. Quantitative and morphological analysis of the trichomes was performed using Image J software (Schneider et al., 2012).

RNA-seq analysis in leaf and isolated stem-trichomes

RNA was extracted from frozen-powdered leaves and isolated stem-trichomes using NucleoSpin® RNA XS kit (MACHEREY-NAGEL, <https://www.mn-net.com>). Starting with 500ng RNA, a poly-A enrichment was performed with NEBNext Poly(A) mRNA Magnetic Isolation Module (New England BioLabs). Next, the NEBNext Ultra II Directional RNA Library Prep Kit and NEBNext Multiplex Oligos (New England BioLabs) were used to generate the RNA-seq libraries according to the manufacturer protocol. The libraries were then assessed for size distribution using a 2200 TapeStation System with Agilent D1000 ScreenTapes (Agilent Technologies) and quantified on a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific) with the NEBNext Library Quant Kit (New England BioLabs). The single end sequencing (1 × 75 bp) of the clustered libraries was performed with NextSeq 500/550 High Output Kit v2.5 (75 Cycles) (Illumina) on a NextSeq 550 Sequencing System (Illumina). Via a Snakemake pipeline (Bliet T. et al., 2021) the reads were first quality-checked and trimmed using fastp (S. Chen et al., 2018) with default settings, then mapped with STAR (Dobin et al., 2013) on SL4.0 tomato

genome (<https://solgenomics.net>) with maximum 10 multi-mappers and 4 mismatches. Finally, read count tables for downstream analyses were generated with featureCounts and annotated according ITAG4.1 SGN annotation (<https://solgenomics.net>). The differential expressed genes (DEG) were identified using the DEseq2 package (Love et al., 2014) setting the threshold for the log₂ fold change > 1 and for the Wald test p-value < 0.05. Gene ontology enrichment analysis was performed using AgriGO (T. Tian et al., 2017) or, together with KEGG pathway enrichment via ShinyGO (Ge et al., 2020; Kanehisa et al., 2021), while STRING was used to predict and visualize putative protein networks (Szkłarczyk et al., 2023).

4.4 Results

Phenotype of a new tomato trichome mutant

To select tomato plants with an aberrant type VI trichomes, the trichomes of 8000 individuals from an EMS-mutagenized population were phenotyped with a stereomicroscope. In the same EMS population, the *myc1* mutant had been previously identified (Xu et al., 2018). A different trichome mutant was selected, missing the glandular cells of type VI glandular trichomes, resulting in a glandular trichome type not observed before on cultivated tomato, and for this reason it was named *glandless* (Figure 1 A-B). Visually, the most evident phenotype of *glandless* plants seemed a reduced density of hairy trichomes on leaves (Figure 1 C-D) and stems (Figure 1 E-F) but not on sepals (Figure 1 G-H).

The *glandless* mutant has aberrant type VI glandular trichomes

To fully investigate the trichome morphology on the *glandless* mutant line, leaf (Figure 2 A-B) and stem surfaces (Figure 2 C-D) were imaged using Cryo-SEM. Architecture and dimensions of non-glandular hairy trichomes of type III, and V were not different between wild-type (WT) and *glandless* on both leaf sides and on stems. Likewise, on *glandless* leaves glandular trichomes of type VII and of the rarely occurring type I were like the WT. Instead, detailed inspection of type VI trichome morphology supported our previous observations as no trichomes resembling the WT (Figure 2 E) could be found on stem and leaves of *glandless* (Figure 2 F). The novel trichome type in this mutant emerges from a single simple epidermal basal cell similarly to type VI, and has a comparable-sized stalk supporting, presumably, a single gland cell. Because of these similarities and the absence of type IV trichome from adult cultivated tomatoes (Glas et al., 2012; Vendemiatti et al., 2017), we hypothesize these trichomes could be morphologically abnormal type VI glandular trichomes. However, the stalk is formed not by one but of two, or sometimes three, cells, suggesting a possible alteration in the cell division pattern. The four gland cells forming type VI trichome head are absent and, in their place, *glandless* type VI trichome heads exhibit a single round-shaped cell. In tomato, the metabolites are secreted by the four gland cells of type VI trichomes to accumulate in the intercellular cavity and can be released when physically damaged by a stretched-out glass Pasteur pipette (J Kortbeek et al., 2023; Tissier et al., 2017a). However, on *glandless*, the single round apical cell could not be ruptured in this manner, suggesting it to be the type VI intermediate cell rather than a gland cell. Overall, these results suggests that a mutation in *glandless* involves a gene regulating type VI trichome development causing the observed anomalous morphology.

Leaf trichome densities are different in *glandless* mutant

To examine whether the *glandless* mutation influences also trichome densities, Cryo-SEM images of both the adaxial and abaxial leaf surfaces were examined. On both the adaxial (AD) and the abaxial (AB) leaf surfaces no difference was observed in the number of type I and III trichomes, but significantly fewer type V trichomes were found on both leaf sides (Figure 3 A, B; Supplemental figure 2). On the AD side, there were significantly more mutant type VI trichomes on *glandless* than normal VI trichomes in the WT but not on the AB side of the leaves (Figure 3 A, B; Supplemental figure 2). Instead, type VII glandular trichomes had lower density on the AB but not on the AD side (Figure 3 A, B; Supplemental figure 2). Overall the trichome density is much reduced in the *glandless* mutant (Figure 3 C) and the number of the different type of trichomes was also changed (Figure 3 D). Altogether, these results indicate that the selected *glandless* line is a new trichome mutant that presents not only altered type VI trichome morphology but also has different type V and type VII densities.

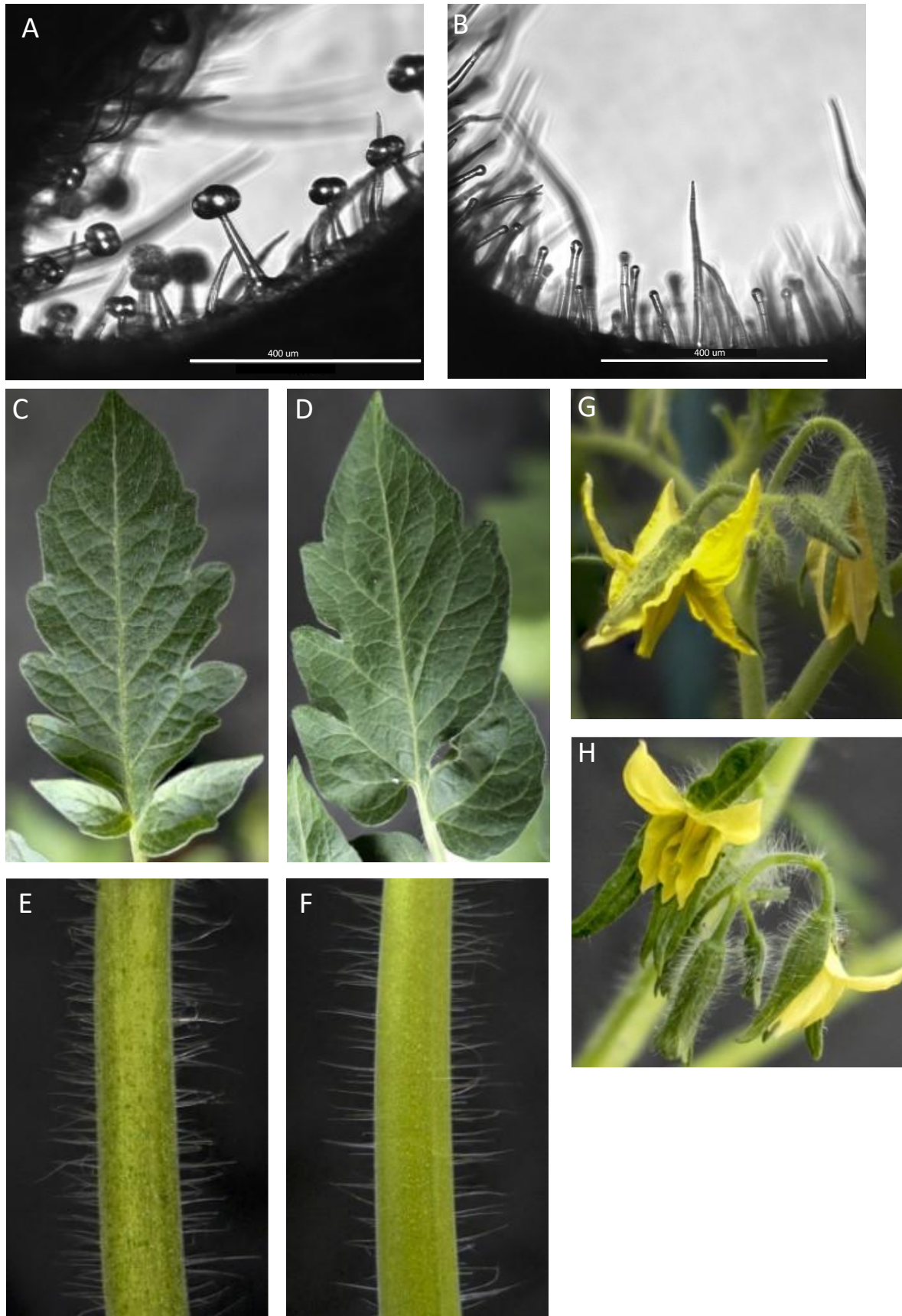


Figure 1 *Phenotype of glandless mutant.* Type VI glandular trichomes as seen with an EVOS inverted light microscope on leaves of wild-type (WT) and (A) on the glandless mutant (B) tomato. Comparison between wild-type (WT) and glandless leaves (C-D) and trichomes visible on respectively wild-type (WT) and glandless stems (E-F) and sepals (G-H).

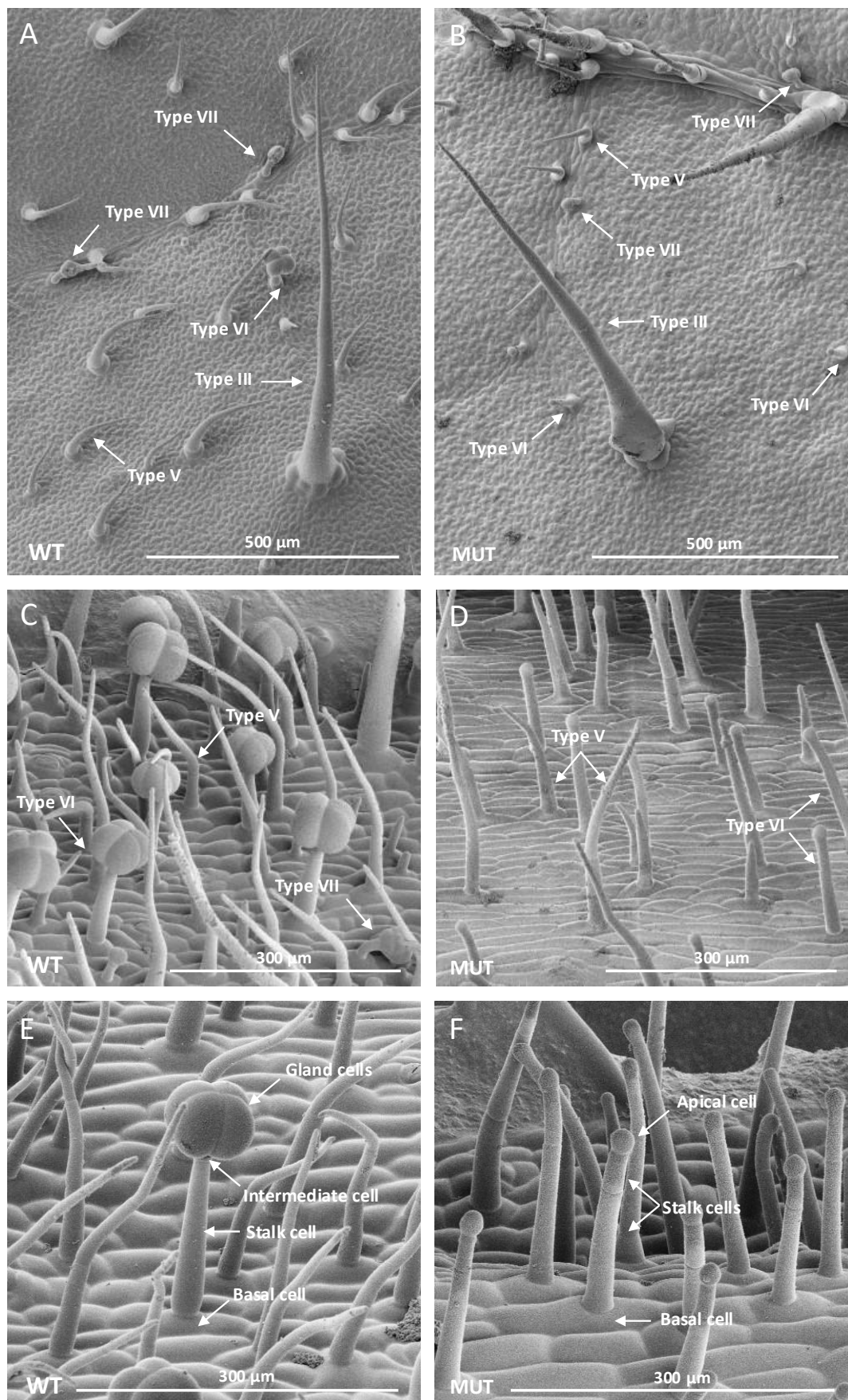


Figure 2 Morphology of glandless trichomes on leaves and stems. Cryo-SEM images of wild-type (WT) and glandless mutant (MUT) leaves (A-B) and stems (C-F). Arrows indicate the different types of trichomes and the cell-types that constitute type VI glandular trichomes.

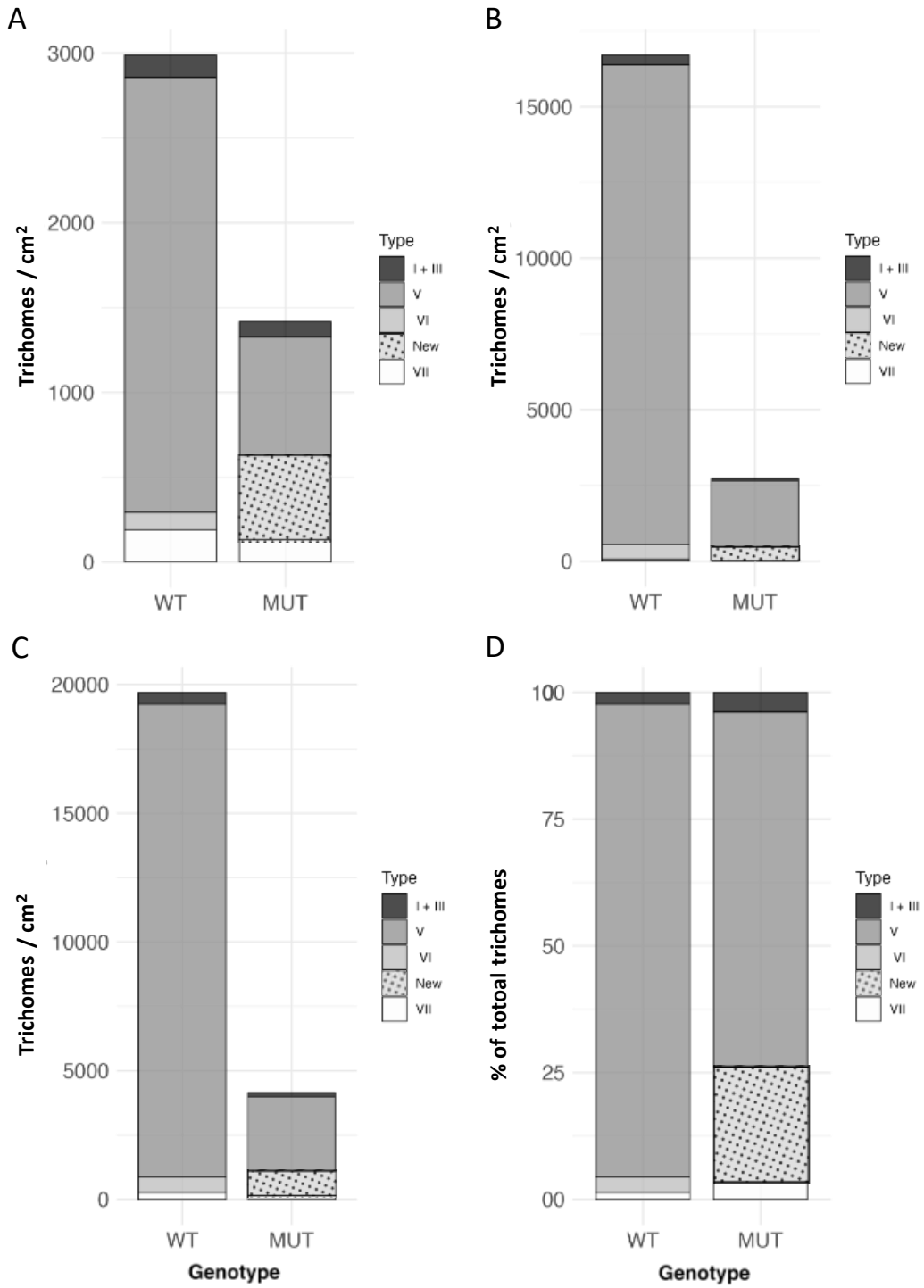


Figure 3 Leaf trichomes densities. Densities of type I and III, type V, type VI, type VII and of the new trichome type on (A) adaxial side, (B) abaxial side and (C) on total leaf surface of wild-type (WT) and glandless mutant (MUT) plants. (D) Percentage of each trichome type of the total leaf trichome density.

In the *glandless* mutant the levels of volatile mono- and sesquiterpenes are reduced

To study whether on the *glandless* mutant the lack of the four glandular cells also impacted the production of volatile terpenoids synthesized and stored in type VI glandular trichomes, monoterpene (α-pinene, 2-carene, α-phellandrene, α-terpinene, D-limonene, β-phellandrene, trans-β-ocimene, terpinolene, linalool) and sesquiterpene (β-caryophyllene and α-humulene) levels were quantified on WT and *glandless* mutant (Figure 4 and Supplemental Figure 3). In the *glandless* mutant all the measured mono- and sesquiterpenes were significantly reduced on both stems (Figure 4 A, B) and leaves (Figure 4 C, D). Similar results were observed also when analysing 200 gland cells manually isolated from type VI trichomes on leaves and the corresponding cell left on the new trichomes in *glandless* (Figure 4 E, F). These results show that the *glandless* mutation affects the levels of volatile mono- and sesquiterpenes suggesting either that *glandless* directly regulates terpene biosynthesis or that the reduction is an indirect effect of the alteration of type VI glandular trichome morphology.

***glandless* is mutated in Solyc09g008810**

The *glandless* mutation was fine-mapped to chromosome 9, specifically to the Solyc09g008810 gene (Figure 5 A). To determine the mutation, the genomic sequence of this gene in the WT and in the *glandless* mutant were cloned and sequenced. The *glandless* allele presented a T to A substitution in position 938 near the 3' end of the first intron. This single nucleotide polymorphism (SNP) introduces a premature acceptor splicing site (AG) that causes a 10 bp frameshift to the coding sequence (CDS) of the gene that results in an early stop codon after a stretch of seven amino acids (Figure 5A). To confirm that this mutation is responsible for the observed trichome phenotype, a functional analysis of the Solyc09g008810 gene was performed using VIGS in *S. lycopersicum* cv. Micro-Tom, also targeting eGFP and *SIPDS1* as negative and positive controls respectively. After 5 weeks, microscopical observations revealed that, compared to the negative control, leaves in which Solyc09g008810 was silenced had aberrant type VI glandular trichomes that resembled the morphology of the ones observed on the *glandless* mutant (Figure 5B). Accordingly, the levels of known mono- and sesquiterpenes were reduced (Figure 5C). Therefore, we concluded that the Solyc09g008810 gene, carrying the SNP site in the *glandless* mutant, is responsible for the type VI glandular trichome phenotype observed on this line. Overall, these results demonstrate that a mutant allele of the Solyc09g008810 gene is responsible for the trichome phenotypes in the *glandless* tomato mutant, suggesting that the encoded protein controls the development of type VI glandular trichomes.

SIHDZ38 is a new candidate regulator of tomato trichomes

The Solyc09g008810 gene is predicted to code for a 242 amino acids long uncharacterized protein with a homeobox domain, widely reported to mediate specific binding to DNA, and a leucine-zipper motif, that is known to mediate formation of protein dimers. This classifies the protein as part of subfamily I of the plant-specific HD-ZIP TF family. In tomato, a total 49 genes belong to it (Hong et al., 2021) and Solyc09g008810 gene encodes SIHDZ38. To gain more insight in its functions, the amino acid sequence of SIHDZ38 was used to find homologs via BLASTp on the NCBI non-redundant protein database. The homology research returned many HD-ZIP-I TFs from various plant species, including hemp, grapevine, poplar, cotton and cucumber (Figure 6A). The alignment of the selected plant homologs of SIHDZ38 revealed a high level of conservation among HD-ZIP I transcription factors, particularly in the regions of the homeobox domain (Q63-A122) and the leucine zipper motif (K123-L157) (Figure 6B). The closest homolog in *Arabidopsis thaliana* is ATHB51 (HOMEBOX 51) also known as ATLMI1 (LATE MERISTEM IDENTITY 1). Remarkably, in *Cucumis sativus* a TF characterized as a regulator of trichome differentiation was found, encoded by the CsGL1 (GLABROUS 1; Csa3G748220) gene, whose mutant alleles named CsGL1, CsMICT (*MICRO-TRICHOME*) and CsTBH (*TINY BRANCHED HAIR*) all show a glabrous phenotype (Q. Li et al., 2015; Pan et al., 2021; Y. Zhang et al., 2021; J.-L. Zhao et al., 2015). The *glandless* allele, due to an early stop codon, encodes for a 40 amino acid long protein that completely lacks both the DNA-binding and the dimerization domains (Figure 6C). These results corroborate our finding that the *glandless* phenotype is caused by the mutation in SIHDZ38.

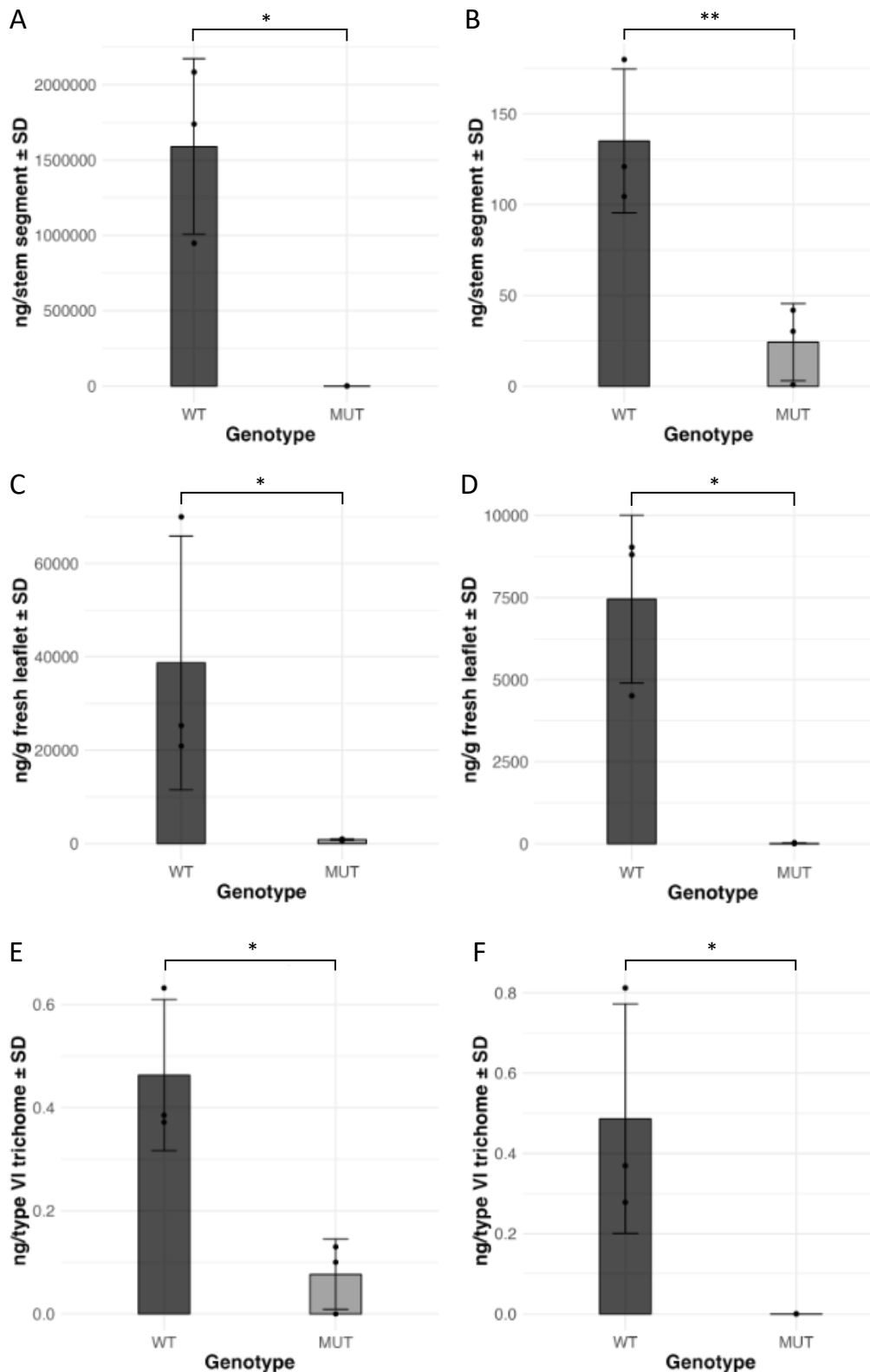


Figure 4 Total known volatile terpenes levels in leaves, stems and isolated type VI trichomes. Total mono- (A, C, E) and sesquiterpene (B, D, F) levels respectively in stems (A, B), leaves (C, D) and isolated type VI trichomes (E, F) of wild-type (WT) and glandless mutant (MUT) tomato plants (n=3). The bars represent the mean values ± standard deviation (SD) of the sum of the levels of target volatile terpenes quantified by GC-MS and normalized by stem length, leaf fresh weight or number of isolated type VI trichomes. Differences in bars are annotated accordingly to the significance levels resulting from independent t-tests after Shapiro-Wilk's normality test and F-test comparison of variances (* p<0.05, ** p<0.01). Significance for non-normally distributed subset was assessed with Wilcoxon non-parametric test.

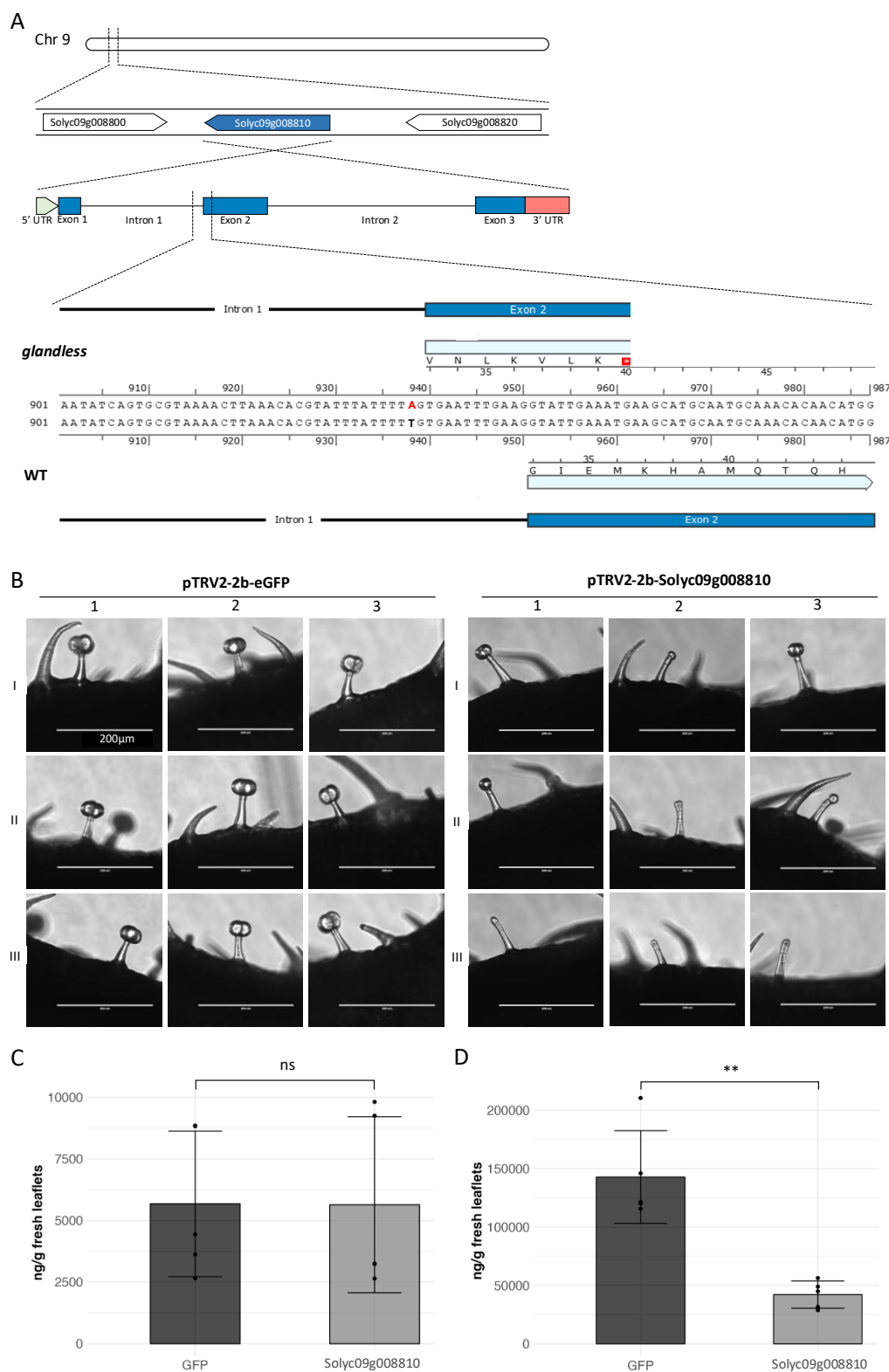


Figure 5 Mapping of *glandless* mutation and validation of phenotype with VIGS. (A) The SNP was mapped on chromosome 9 in the *Solyc09g008810* gene. Compared to the wild-type sequence (WT) in *glandless* the T938A substitution generated a premature splicing acceptor site causing a frameshift and an early stop codon. 5 weeks after VIGS, targeting *Solyc09g008810*, or GFP as negative control, (B) an aberrant type VI trichome phenotype was observed with a microscope, on 3 different leaves (I-III) of 3 independent plants (1-3). Additionally, on the same plants the total levels \pm standard deviation (SD) of known volatile monoterpenes (C) and sesquiterpenes (D) were quantified with GC-MS (n=4). Differences in bars are annotated accordingly to the significance levels resulting from independent t-tests after Shapiro-Wilk's normality test and F-test comparison of variances (** $p < 0.01$, ns non-significant).

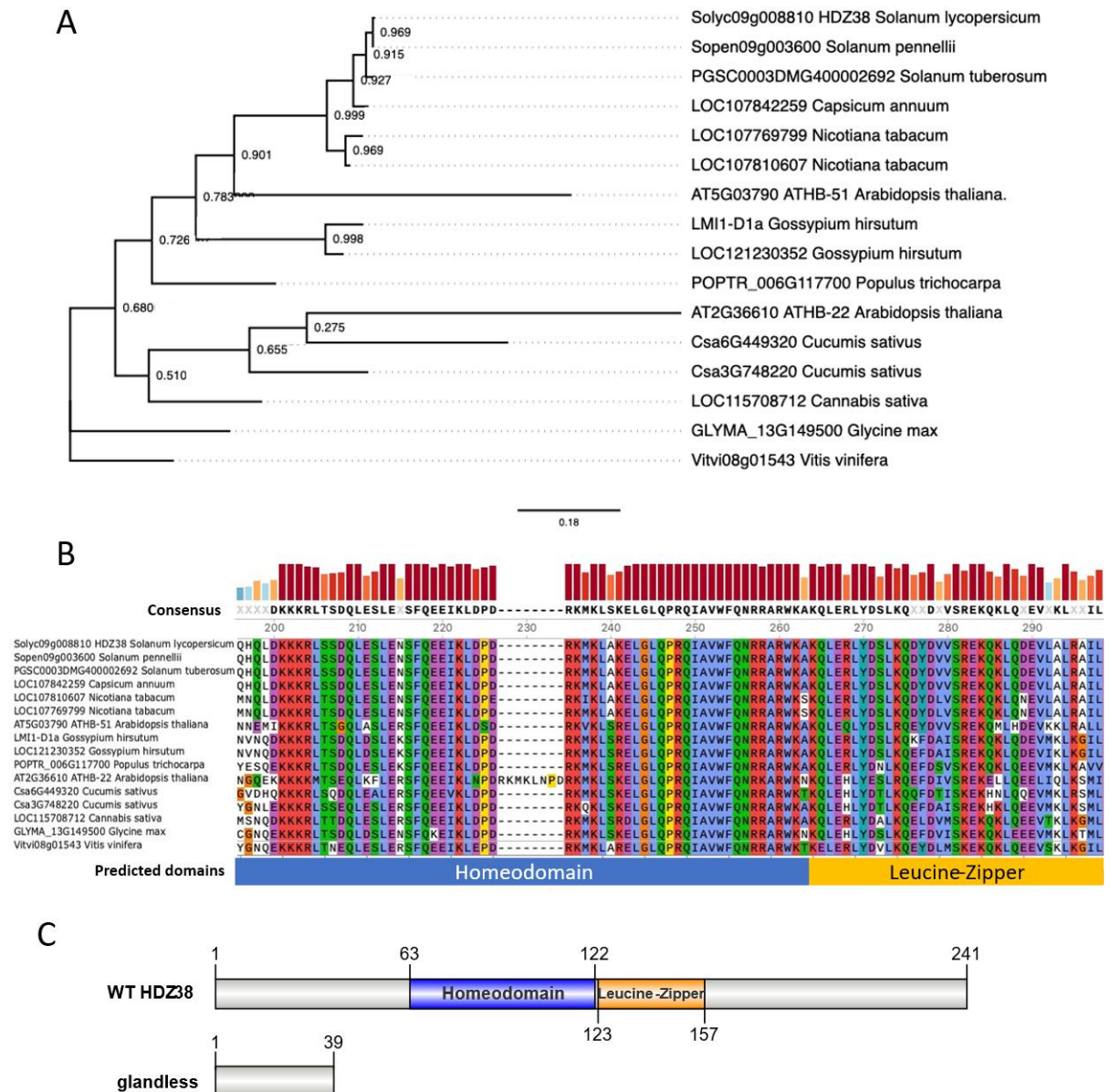


Figure 6 Phylogenetic relationship of SLHDZ38 homologs in selected plants. (A) Putative homologs of SLHDZ38 protein were selected from BLASTp (NCBI) results, aligned with TCOFFEE algorithm and used to construct a phylogenetic tree in PhyML with the maximum-likelihood method. Values on the branches indicate the supporting bootstrap values of 500 replications. (B) Detail of the multi-sequence alignment shows the high conservation of the Homeodomain and the Leucine-zipper motifs among selected homologs. (C) The truncated protein encoded by the glandless allele of lacks both the functional domains of the wild-type protein (WT).

SIHDZ38 *glandless* mutation alters the expression of trichome-specific regulatory and metabolic genes

With the aim to study the impact of the mutation at the transcriptional level, RNA sequencing was performed on leaves and isolated stem trichomes of WT and *glandless* mutant (Supplemental table 2). The expression of genes already known to be involved in trichome development and in type VI trichome-specific metabolism was analysed. In leaves, seven mono- and sesquiterpene synthase genes, were less expressed (Table 1). Additionally, reduced expression was found for the JA signalling inhibitors *SIJAZ2* and *SIJAZ4* that control *SIMYC1* and its interaction with *SIWO*, essential in the regulation of type VI trichome development and terpene metabolism (Hua et al., 2020; Xu et al., 2018). Slightly but significantly lower transcript levels in *glandless* leaves were also observed for *SIMTR1* and two HD-ZIP TFs, *SILN* and *SIHD8*, all three involved in trichome development. Also *SIGCR1*, one of the two recently identified MYB-like TF that inhibit the formation of trichome gland cells (Chang et al., 2024) was found to have lower expression levels in *glandless*. These results support the double role for *SIHDZ38* as being involved – directly or indirectly by modulating the levels of other TFs – in controlling volatile mono- and sesquiterpenes biosynthesis and regulating trichome development. Many terpene synthases were among the lowest expressed genes also in isolated stem trichomes, including the type VI glandular trichome-specific *SITPS12* and *SITPS5* (Table 2). Dramatically reduced were also the transcriptional levels of *SIEOT1*, *SIEOT2*, *SIMYC1* and *SISCL3*, that share a particularly high expression in trichomes and function as transcriptional regulators of terpene biosynthetic pathway genes (E. Spyropoulou, 2012; E. A. Spyropoulou et al., 2014a, 2014b; Xu et al., 2018; J. Xu, 2023; Yang et al., 2021). In the isolated stem trichomes of *glandless*, among factors known to be involved in trichome development *SISH*, *SIMIXTA*-like and the HD-ZIP IV TFs *SIHD8* and *SICD2* had higher transcript levels. Moreover, *SIWO* and two of its downstream targets in hairy-trichome regulation, *SIMX1* and *SIWOX3b*, were all upregulated. Accordingly, *SIWO* negative regulator *SIMTR1* showed lower expression, while its homologs *SIMTR2* and *SIMTR3* had higher expression. Lastly, the JA signalling inhibitors *SIJAZ5*, *SIJAZ6* and *SIJAZ7* were also more expressed in *glandless* than in WT. In both leaves and stem-trichomes, *SITOEb1* and *SIGCR1*, two recently characterized TFs that regulate trichome gland cells formation via controlling downstream *SILFS* (Chang et al., 2024). Altogether, the simultaneous differential expression of trichome-specific metabolic enzymes and regulatory transcription factors in isolated stem trichomes represent another piece of evidence that *SIHDZ38* is involved in trichome development and specialized metabolism.

SIHDZ38 controls type VI trichome initiation

To further investigate the role of *SIHDZ38* in trichome development we generated CRISPR-Cas9 knockout lines in the tomato cultivar Micro-Tom (Supplemental Figure 1). Three independent T1 plants, each homozygous for a different nucleotide edit were selected and named CC-14-1, CC-14-2 and CC-14-3 (Supplemental Figure 4). All three different mutations result in early stop codons that determine truncated proteins missing both functional domains (Supplemental Figure 5). Remarkably, already during the screening of plants of the T1 generation, it was observed with the stereomicroscope that plants CC-14-1, CC-14-2 and CC-14-3 completely missed type VI trichomes on both leaves and stem, while the other trichome types were morphologically unaltered (Figure 7). Subsequently, trichome densities and known volatile terpenes were quantified on multiple plants from the T2 generation of line CC-14-1, CC-14-2 and CC-14-3.

As observed in the previous generation, when compared to the WT, type VI glandular trichomes were completely absent on both sides of the leaves from all three *SIHDZ38* KO lines (Figure 8A-B). Accordingly, when compared to the WT, on CC-14-1, CC-14-2 and CC-14-3 leaves the levels of known monoterpenes (Figure 9A, Supplemental Figure 6) and sesquiterpenes (Figure 9B, Supplemental Figure 6) were dramatically reduced. None of other trichome types were morphologically altered by knocking-out *SIHDZ38*. Overall, these results confirm our previous finding that *SIHDZ38* transcription factor is involved in the regulation of type VI trichome development. Additionally, the knockout lines show that the HD-ZIP TF is also essential for the initiation of type VI glandular trichomes.

Solyc ID	Gene	log2FC	p-value	Class	Family	Annotation
Solyc06g059930	TPS9	-10.34	2.9E-15		TPS	Sesquiterpene synthase (Germacrene), trichome-specific, cytosol
Solyc08g005710	TPS41	-7.91	7.9E-12		TPS	Terpene synthase, mitochondrial
Solyc01g105850	TPS1	-6.51	2.6E-05		TPS	Monoterpene synthase (Camphene/tricyclene), chloroplastic
Solyc10g005390	TPS39	-5.19	3.4E-02		TPS	Monoterpene synthase (Linalool/nerolidol), cytosolic
Solyc12g006570	TPS17	-4.65	3.2E-06		TPS	Sesquiterpene synthase (Valencene/bisabolene), cytosolic
Solyc08g005670	TPS10	-2.94	3.7E-02		TPS	Monoterpene synthase (Phellandrene), cytosolic
Solyc03g006550	TPS46	-2.40	2.2E-09		TPS	Putative geranylinalool synthase
Solyc08g005720	TPS18	-2.27	3.2E-03		TPS	Terpene synthase, mitochondrial
Solyc12g049400	JAZ4	-2.55	7.0E-13	TF	Tify	Jasmonate ZIM-domain protein, Tify
Solyc12g009220	JAZ2	-1.20	1.3E-06	TF	Tify	Jasmonate ZIM-domain protein 1
Solyc02g076670	GCR1	-1.03	6.7E-06	TF	GARP-G2-like	Myb domain, Homeodomain-like protein
Solyc10g083140	MTR1	-0.77	1.5E-03		Cyclin	Hypothetical protein
Solyc03g031760	LN	-0.64	3.6E-05	TF	HD-ZIP	Homeobox-leucine zipper protein ROC2
Solyc03g098200	HDZ8	-0.56	2.7E-02	TF	HD-ZIP	Homeobox-leucine zipper protein HDG12
Solyc04g049800	TOE1b	0.48	3.76E-02	TF	ARF2/ERF	AP2-like ethylene-responsive transcription factor
Solyc02g076670	GCR1	0.72	7.84E-04	TF	GARP-G2-like	MYB-like domain, Homeodomain-like protein

Table 1 Differentially expressed trichome genes in leaves of glandless mutant

Solyc ID	Gene	log2FC	p-value	Class	Family	Annotation
Solyc10g005390	TPS39	-8.66	2.90E-14		TPS	Monoterpene synthase (Linalool/nerolidol), cytosolic
Solyc08g005720	TPS18	-8.64	1.72E-17		TPS	Terpene synthase, mitochondrial
Solyc01g105850	TPS1	-8.41	5.65E-13		TPS	Monoterpene synthase (Camphene/tricyclene), chloroplastic
Solyc08g005670	TPS10	-8.41	7.85E-10		TPS	Monoterpene synthase (Phellandrene), cytosolic
Solyc01g101170	TPS31	-8.38	4.20E-12		TPS	Sesquiterpene synthase (Viridiflorene), cytosolic
Solyc06g059930	TPS9	-8.38	6.90E-08		TPS	Sesquiterpene synthase (Germacren), trichome-specific, cytosol
Solyc07g008690	TPS16	-8.28	3.93E-20		TPS	Sesquiterpene synthase
Solyc08g005710	TPS41	-8.02	1.85E-13		TPS	Terpene synthase, mitochondrial
Solyc08g005640	TPS21	-5.60	5.40E-65		TPS	Terpene synthase, plastidic
Solyc01g105890	TPS5	-5.52	7.16E-17		TPS	Monoterpene synthase (Linalool), trichome-specific, plastidic
Solyc01g101210	TPS35	-5.35	8.40E-03		TPS	Terpene synthase, cytosolic
Solyc01g105920	TPS7	-2.90	1.49E-04		TPS	Monoterpene synthase (Beta myrcene/limonene), plastidic
Solyc12g006570	TPS17	-1.71	2.67E-10		TPS	Sesquiterpene synthase (Valencene/bisabolene), cytosolic
Solyc02g062400	EOT1	-3.96	2.92E-79	TF	SRS	Expression of terpenoids 1
Solyc08g005050	MYC1	-3.66	8.94E-55	TF	bHLH	bHLH Transcription factor MYC1
Solyc03g033680	EOT2	-2.45	1.60E-21	TF	SRS	Expression of terpenoids 2
Solyc12g099900	SCL3	-2.08	5.06E-17	TF	GRAS	Scarecrow-like 3
Solyc09g008810	HDZ38	-1.56	5.36E-04	TF	HB-HD-ZIP	Homeobox-leucine zipper protein ATHB-22
Solyc02g076670	GCR1	-1.03	3.17E-04	TF	GARP-G2-like	MYB-like domain, Homeodomain-like protein
Solyc10g083140	MTR1	-0.56	8.48E-03		Cyclin	hypothetical protein
Solyc09g014980	DT1	0.34	4.72E-03	TF	SCAR	Protein SCAR4
Solyc03g098200	HDZ8	0.41	1.15E-03	TF	HB-HD-ZIP	Homeobox-leucine zipper protein HDG12
Solyc01g091630	CD2	0.52	8.96E-07	TF	HB-HD-ZIP	Cutin deficient 2
Solyc03g118540	JAZ5	0.66	4.43E-02	TF	Tify	Jasmonate ZIM domain protein
Solyc02g088190	MIXTA-like	0.71	4.88E-07	TF	MYB	MYB transcription factor
Solyc02g076670	GCR1	0.72	9.43E-05	TF	GARP-G2-like	Myb domain-, Homeodomain-like protein
Solyc04g049800	TOE1b	0.79	1.40E-02	TF	AP2/ERF	AP2/ERF transcription factor
Solyc01g005440	JAZ6	0.99	3.38E-07	TF	Tify	Jasmonate ZIM-domain protein
Solyc06g073990	MTR2	0.99	7.18E-06		Cyclin	Hypothetical protein
Solyc02g080260	WO	1.01	2.49E-11	TF	HB-HD-ZIP	Woolly
Solyc01g007870	MTR3	1.09	1.88E-07		Cyclin	hypothetical protein
Solyc11g011030	JAZ7	1.11	5.60E-03	TF	Tify	Pto-responsive gene 1
Solyc01g010910	MX1	1.21	3.45E-03	TF	MYB	MYB transcription factor subfamily 9
Solyc11g072790	WOX3b	1.90	1.67E-04	TF	HB-WOX	WOX3b
Solyc10g078990	SH	4.17	4.23E-04	TF	C2H2	Zinc finger protein 6

Table 2 Differential expressed trichome genes in isolated stem-trichomes of glandless mutant

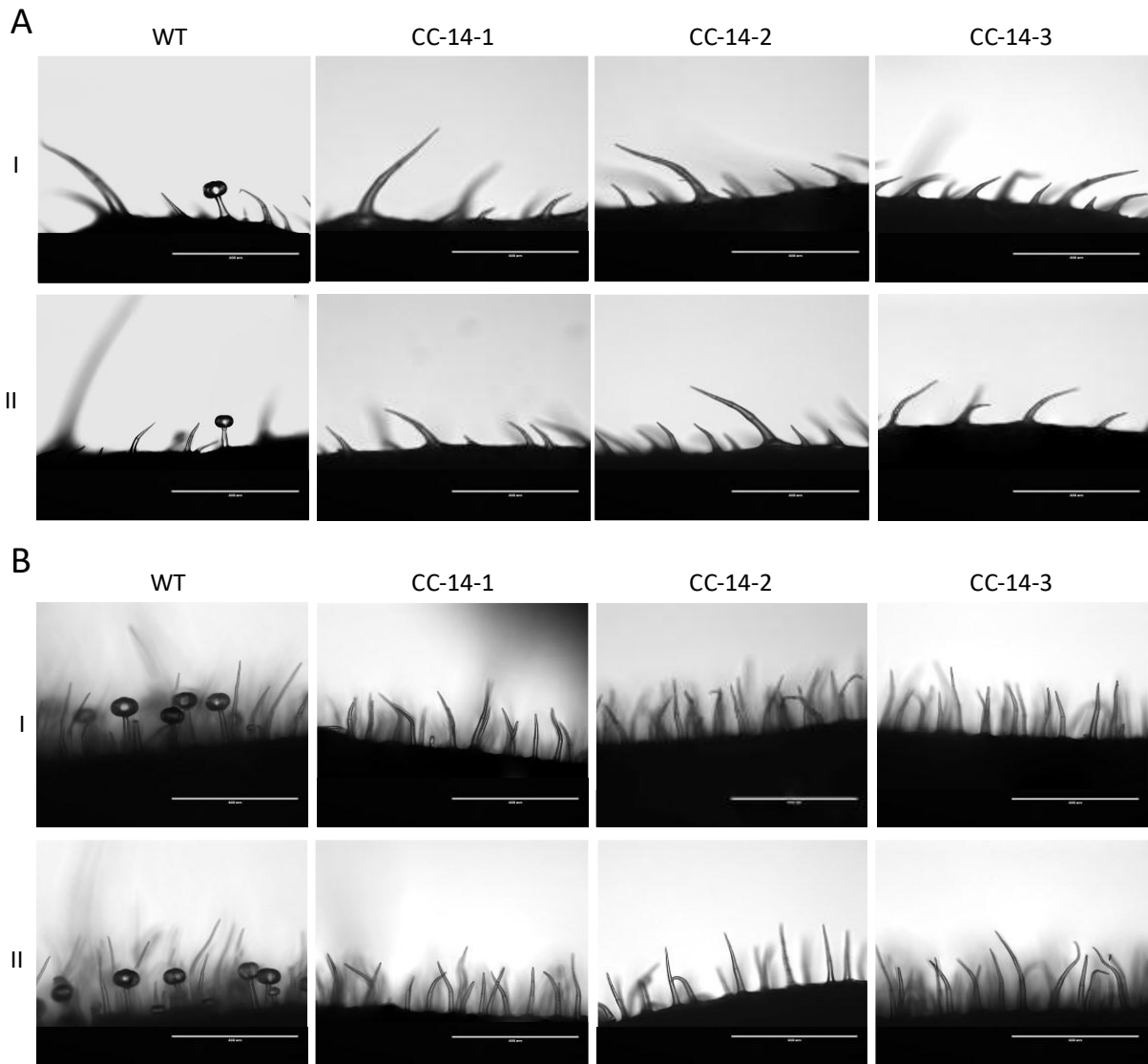
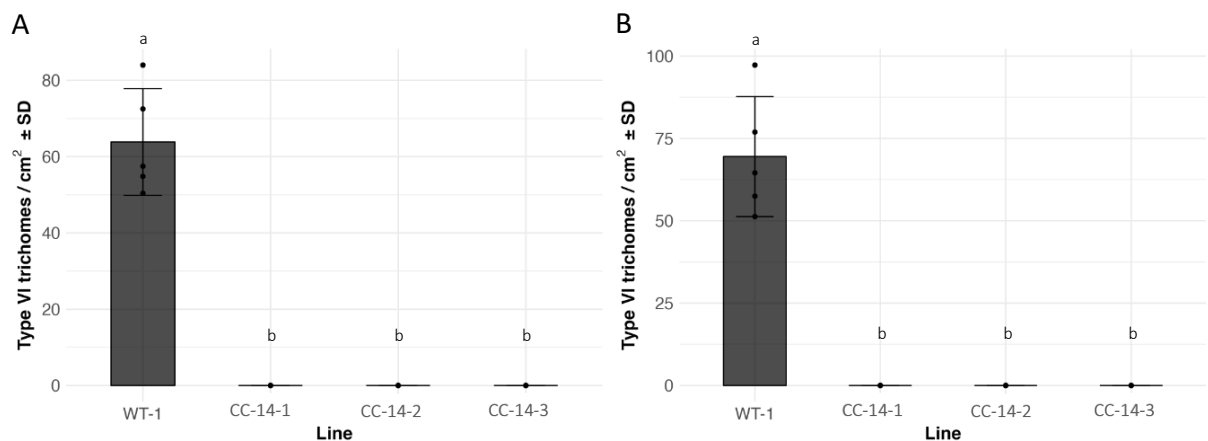


Figure 7 Type VI glandular trichomes on leaves and stem of *SIHDZ38* gene-edited T1 lines. Type VI trichomes as observed with an EVOS inverted light microscope on two different (I-II) leaves (A) and stems (B) of wild-type (WT) and three independent



CRISPR-Cas9 (CC) T2-lines.

Figure 8 Leaf type VI trichome density of *SIHDZ38* KO lines. Densities of type VI glandular trichomes \pm standard deviation (SD) on the (A) adaxial side and (B) abaxial side of leaves ($n = 5$) from wild-type (WT-1) and three independent T2 CRISPR-Cas9 *SIHDZ38* gene-edited lines (CC-14-1, CC-14-2, CC-14-3). Significant differences in bars are annotated with different letters according to Fisher's LSD test after one-way ANOVA ($p < 0.05$).

4.5 Discussion

This study revealed that a mutant allele of SIHDZ38, a novel HD-ZIP subfamily I transcription factor, is responsible for the *glandless* tomato mutant phenotype. Additionally, we demonstrated that HDZ38 is essential for the development of type VI glandular trichomes and that it is also involved in the regulation of the densities of type VII glandular and type V non-glandular trichomes.

SIHDZ38 is a new regulator of type VI glandular trichome development

Tomato has become the model plant to study the regulation of development and specialized metabolism of multicellular glandular trichomes in horticultural crops. Among the proteins characterized so far as regulators of these two processes, some belong to the subfamily IV HD-ZIP TF family, such as SIWO and SICD2. In this study we characterized a novel tomato mutant, *glandless*, showing new type of trichomes but missing type VI glandular trichomes. The absence of mono- and sesquiterpenes in *glandless* plants and the drastic downregulation of many genes typically highly expressed in type VI glandular trichomes lead us to conclude that the new trichome type is a morphologically aberrant type VI missing the gland head. By mapping the mutation, we found that a subfamily I HD-ZIP TF, SIHDZ38, underlies the *glandless* mutation as verified by VIGS that generated an identical mutant trichome phenotype. Overall, these results demonstrate that SIHDZ38 is a regulator of type VI glandular trichome development, specifically of the glandular head formation. The cucumber homolog of HDZ38, CsGL1, whose mutant exhibits aborted glandular trichome development, is expressed during the developmental stage where the glandular head formation occurs (Dong et al., 2022), supporting our conclusion of a similar role for SIHDZ38 in tomato. Many TFs of the HD-ZIP subfamily I, including SIHB2 in tomato, have been previously shown to regulate auxin signaling and transport, and developmental responses to light drought, salt, cold and heavy metal stresses (S. Gong et al., 2019; Hu et al., 2017). However, SIHDZ38 of this subfamily clearly has a role in glandular trichome development.

A putative role of SIHDZ38 in type VI trichome initiation

Interestingly, knocking out SIHDZ39 via CRISPR-Cas9 resulted in the complete disappearance of these aberrant trichomes and thus of type VI glandular trichomes altogether. This additional evidence confirms our hypothesis that the *glandless* trichomes are indeed aberrant type VI trichomes. For now, we can only speculate why the *glandless* mutant allele of SIHDZ38 and its knockdown via VIGS do not lead to the disappearance of type VI trichomes as observed in the knockout. This could be because in the EMS mutant the aberrant splice site, introduced by the mutation, is only partially used, and thus the plant still expresses some full-length mRNA. Therefore, some of the wild-type SIHDZ38 protein could be translated up to a concentration sufficient to induce the initial emergence of type VI trichomes but not enough to later determine the formation of the gland cells. We also suggest such a dose-dependent effect for the *glandless* phenotype observed on the plants where *SIHDZ38* is silenced with VIGS. Likewise, a similar dose-dependent regulatory mechanism was recently proposed for SIWO, a HD-ZIP IV TF involved in trichome development (M. Wu et al., 2023). Nevertheless, the different phenotypes obtained with the knockdown of *SIHDZ38* and its knockout, provide useful additional information about its function. In fact, this finding suggests that the role of SIHDZ38 in tomato is not only to control the gland head formation but more extensively to regulate the initiation of type VI glandular trichomes. Work on cucumber is in support of this hypothesis, where the homolog *CsGL1* starts to be expressed in the epidermal cell during its expansion and first division leading to trichome formation (Dong et al., 2022).

SIHDZ38 is part of the tomato trichome regulatory network

In this study we analysed how the *glandless* mutation affects, in leaves and isolated stem trichomes, the transcript levels of terpene biosynthetic enzymes and known transcription factors involved in trichome development. Our findings highlight, as expected, that the absence of gland cells from type VI glandular trichomes in *glandless* determine that mono- and sesquiterpene synthases are hardly expressed in the residual trichomes. Additionally, many TFs that are normally highly expressed in gland

cells of type VI trichomes have lower transcript levels in isolated trichomes of *glandless* than in the WT. Some of these are involved in trichome development, like *SIMTR1* and *SIHD8*, whereas others participate in regulating specialized metabolism, such as *EOT1* and *EOT2*, or both processes, such as *SIMYC1* and *SISCL3*. In leaves the situation is somewhat peculiar: in spite of the fact that *SIHDZ38* is hardly expressed in WT leaf tissue – other than in trichomes – the *glandless* mutation still reduces the expression of *SIMTR1* and *SIHD8*, genes known to be involved in trichome development. This suggests that *SIHDZ38* regulates both trichome development and specialized metabolism as previously also suggested for *SIMYC1* (Xu et al., 2018).

It has been recently suggested that higher *SIWO* levels preferentially activate *SIMX1* and *SIWOX3* expression, promoting the differentiation of hairy trichomes and inhibiting glandular trichomes (M. Wu et al., 2023). While in isolated trichomes of the *glandless* mutant the transcript levels of these three TFs are all higher, their expression is unaltered in leaves. Here, the number of type I and III remained unaltered whereas the densities of type V and VII trichomes were severely reduced (Supplemental figure 2). Conversely, the density of the aberrant type VI glandular trichomes was higher in the *glandless* mutant. Therefore, it could be that, in leaves, *SIHDZ38* promotes the initiation of type VI and represses that of type V and VII without involving the *WO-WOX3b-MX1* module. Although our data are not sufficient to completely explain the *glandless* phenotype, they nevertheless indicate that a higher *SIWO* expression does not increase significantly hairy trichome numbers but instead reduces type V density when *SIHDZ38* is mutated. Moreover, higher levels of *SIWO* in *glandless* do lead to an increase in type VI but not of type VII trichomes (Figure 3). Both these findings may indicate another level of fine-tuning of the *SIWO*-dose dependent regulatory mechanism (M. Wu et al., 2023), or may indicate that the concentration of *WO* protein is mostly controlled at post-transcriptional level and the transcriptional regulation by *SIHDZ38* may have a different or later role.

To be further investigated is also the interplay between JA signalling and *SIHDZ38*. It has been shown that JA can regulate tomato trichome initiation, development, and also specialized metabolites biosynthesis (Boughton et al., 2005; Hua et al., 2021; L. Li et al., 2004; Xu et al., 2018; Yan et al., 2013; Yang et al., 2021; Yoshida et al., 2009). Previous studies suggested that *SIJAZ2* inhibits the functionality of the *SIWO-SIMYC1* module in activating terpenes biosynthesis in type VI glandular trichomes, via a competitive binding mechanism (Hua et al., 2020). Additionally, the over-expression of *SIJAZ2* caused down-regulation of *SIWO* and *SIMTR1* in stem epidermis and decreased the abundance of stem trichomes (Yu et al., 2018). *SIJAZ4*, which shows its highest expression in trichomes, inhibits via protein-protein interaction the activity of *SIMYC1* as activator of terpene biosynthesis (Hua et al., 2020) and of *SIHD8* responsible of JA-induced trichome-elongation (Hua et al., 2021). Accordingly, *SIJAZ4* over-expression resulted in shorter trichomes (Hua et al., 2021). The differential gene expression analysis for the *glandless* mutant showed that, in leaves, genes coding for two JA signalling inhibitors, *SIJAZ2* and *SIJAZ4*, have lower expression than in WT plants. This suggests that *SIHDZ38* maintains *SIJAZ2* and *SIJAZ4* expression levels in absence of JA, consequently contributing to stabilize *SIWO* and *SIMTR1* levels and hence trichome initiation, and to preserve the inhibition of *SIHD8* and the *SIWO-SIMYC1* module activity. Interestingly, in stem trichomes, no difference was observed for *SIJAZ2* and *SIJAZ4* levels but *SIJAZ5*, *SIJAZ6* and *SIJAZ7* were higher expressed than in the WT. This suggests a different role for *SIHDZ38* related to JA signalling in stem-trichomes, where it seems to act as negative regulator of *SIJAZ5*, *SIJAZ6* and *SIJAZ7*.

Overall, our results point once more to the very intricate regulatory network of TFs that determines the developmental fate of trichomes in tomato and the regulation of their specialized metabolism. Accordingly, it remains difficult to determine the precise role of *SIHDZ38* in this network and more detailed studies focusing on protein-protein interactions with other known TFs or on binding and activation of their promoters are necessary for this goal. We suggest *SIHDZ38* to be located high up in hierarchy in this network as it appears from the differential expression of other master regulators like

SIHD8 (Hua et al., 2021), SIMTR1 (S. H. Gao et al., 2017; M. Wu et al., 2023; Yang, Li, Zhang, Luo, et al., 2011) and, specifically in stem trichomes, SIWO (Hua et al., 2020; D. Tian et al., 2012; Yang, Li, Zhang, Wang, et al., 2011), SISH (Chun et al., 2021; Hua et al., 2022; R. Li et al., 2021; Zheng et al., 2022) and SIMYC1 (Hua et al., 2020; M. Wu et al., 2023; Xu et al., 2018). To further expand our comprehension on the role of SIHDZ38 in the trichome-regulatory network and validate some of the suggested hypotheses, further studies should also investigate the effect of *SIHDZ38* overexpression on the regulatory network of TFs and on trichomes densities and morphology.

4.6 Acknowledgments

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4.7 Author contributions to the article

PZ carried out the experiments, PZ and RS, and MH designed the research. PZ, wrote the manuscript and RS and MH edited it.

4.8 Bibliography

- Abdullah, M., Cheng, X., Cao, Y., Su, X., Manzoor, M. A., Gao, J., Cai, Y., & Lin, Y. (2018). Zinc finger-homeodomain transcriptional factors (ZHDs) in upland cotton (*Gossypium hirsutum*): Genome-wide identification and expression analysis in fiber development. *Frontiers in Genetics, 9*(OCT), 329913. <https://doi.org/10.3389/FGENE.2018.00357/BIBTEX>
- Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science, 196*, 67–76. <https://doi.org/https://doi.org/10.1016/j.plantsci.2012.07.014>
- Akhtar, T. A., Matsuba, Y., Schauvinhold, I., Yu, G., Lees, H. A., Klein, S. E., & Pichersky, E. (2013). The tomato cis-prenyltransferase gene family. *The Plant Journal, 73*(4), 640–652. <https://doi.org/10.1111/TPJ.12063>
- Amack, S. C., & Antunes, M. S. (2020). CaMV35S promoter – A plant biology and biotechnology workhorse in the era of synthetic biology. *Current Plant Biology, 24*, 100179. <https://doi.org/10.1016/J.CPB.2020.100179>
- Balcke, G. U., Bennewitz, S., Bergau, N., Athmer, B., Henning, A., Majovsky, P., Jiménez-Gómez, J. M., Hoehenwarter, W., & Tissier, A. (2017). Multi-Omics of Tomato Glandular Trichomes Reveals Distinct Features of Central Carbon Metabolism Supporting High Productivity of Specialized Metabolites. *The Plant Cell, 29*(5), 960–983. <https://doi.org/10.1105/tpc.17.00060>
- Bar, M., & Shtein, I. (2019). Plant trichomes and the biomechanics of defense in various systems, with Solanaceae as a model. *Botany, 97*(12), 651–660. <https://doi.org/10.1139/cjb-2019-0144>
- Ben-Israel, I., Yu, G., Austin, M. B., Bhuiyan, N., Auldridge, M., Nguyen, T., Schauvinhold, I., Noel, J. P., Pichersky, E., & Fridman, E. (2009). Multiple Biochemical and Morphological Factors Underlie the Production of Methylketones in Tomato Trichomes. *Plant Physiology, 151*(4), 1952–1964. <https://doi.org/10.1104/pp.109.146415>
- Bennewitz, S., Bergau, N., & Tissier, A. (2018). QTL Mapping of the Shape of Type VI Glandular Trichomes in Tomato. *Front Plant Sci, 9*, 1421. <https://doi.org/10.3389/fpls.2018.01421>
- Bensen, R. J., & Zeevaart, J. A. D. (1990). Comparison of ent-kaurene synthetase A and B activities in cell-free extracts from young tomato fruits of wild-type and gib-1, gib-2, and gib-3 tomato plants. *Journal of Plant Growth Regulation, 9*, 237–242.
- Bergau, N., Bennewitz, S., Syrowatka, F., Hause, G., & Tissier, A. (2015). The development of type VI glandular trichomes in the cultivated tomato *Solanum lycopersicum* and a related wild species *S. habrochaites*. *BMC Plant Biol, 15*(1), 289. <https://doi.org/10.1186/s12870-015-0678-z>
- Besser, K., Harper, A., Welsby, N., Schauvinhold, I., Slocombe, S., Li, Y., Dixon, R. A., & Broun, P. (2009). Divergent regulation of terpenoid metabolism in the trichomes of wild and cultivated tomato species. *Plant Physiology, 149*(1), 499–514.
- Bickford, C. P. (2016). Ecophysiology of leaf trichomes. *Functional Plant Biology, 43*(9), 807–814. <https://doi.org/10.1071/FP16095>

- Bleeker, P. M., Diergaarde, P. J., Ament, K., Schütz, S., Johne, B., Dijkink, J., Hiemstra, H., De Gelder, R., De Both, M. T. J., Sabelis, M. W., Haring, M. A., & Schuurink, R. C. (2011). Tomato-produced 7-epizingiberene and R-curcumenone act as repellents to whiteflies. *Phytochemistry*, *72*(1), 68–73. <https://doi.org/10.1016/J.PHYTOCHEM.2010.10.014>
- Bleeker, P. M., Mirabella, R., Diergaarde, P. J., VanDoorn, A., Tissier, A., Kant, M. R., Prins, M., de Vos, M., Haring, M. A., & Schuurink, R. C. (2012). Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. *Proceedings of the National Academy of Sciences*, *109*(49), 20124–20129. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3523864/pdf/pnas.201208756.pdf>
- Bleeker, P. M., Spyropoulou, E. A., Diergaarde, P. J., Volpin, H., De Both, M. T. J., Zerbe, P., Bohlmann, J., Falara, V., Matsuba, Y., Pichersky, E., Haring, M. A., & Schuurink, R. C. (2011). RNA-seq discovery, functional characterization, and comparison of sesquiterpene synthases from *Solanum lycopersicum* and *Solanum habrochaites* trichomes. *Plant Molecular Biology*, *77*(4–5), 323–336. <https://doi.org/10.1007/s11103-011-9813-x>
- Blik T., Chouaref J., van der Kloet F., & Galland M. (2021). *RNA-seq analysis pipeline (version 0.3.7)*. <https://doi.org/10.5281/ZENODO.4707140>
- Bollier, N., Gonzalez, N., Chevalier, C., & Hernould, M. (2022). Zinc Finger-Homeodomain and Mini Zinc Finger proteins are key players in plant growth and responses to environmental stresses. *Journal of Experimental Botany*, *73*(14), 4662–4673. <https://doi.org/10.1093/jxb/erac194>
- Bosch, M., Wright, L. P., Gershenzon, J., Wasternack, C., Hause, B., Schaller, A., & Stintzi, A. (2014). Jasmonic acid and its precursor 12-oxophytodienoic acid control different aspects of constitutive and induced herbivore defenses in tomato. *Plant Physiology*, *166*(1), 396–410. <https://doi.org/10.1104/pp.114.237388>
- Boughton, A. J., Hoover, K., & Felton, G. W. (2005). Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. *Journal of Chemical Ecology*, *31*(9), 2211–2216. <https://doi.org/10.1007/S10886-005-6228-7>
- Chang, J., Wu, S., You, T., Wang, J., Sun, B., Xu, B., Xu, X., Zhang, Y., & Wu, S. (2024). Spatiotemporal formation of glands in plants is modulated by MYB-like transcription factors. *Nature Communications*, *15*(1), 2303. <https://doi.org/10.1038/s41467-024-46683-0>
- Chang, J., Yu, T., Yang, Q., Li, C., Xiong, C., Gao, S., Xie, Q., Zheng, F., Li, H., Tian, Z., Yang, C., & Ye, Z. (2018). Hair, encoding a single C2H2 zinc-finger protein, regulates multicellular trichome formation in tomato. *Plant Journal*, *96*(1), 90–102. <https://doi.org/10.1111/tpj.14018>
- Channarayappa, Shivashankar, G., Muniyappa, V., & Frist, R. H. (1992). Resistance of *Lycopersicon* Species to Bemisia-Tabaci, a Tomato Leaf Curl Virus Vector. *Canadian Journal of Botany-Revue Canadienne De Botanique*, *70*(11), 2184–2192.
- Chen, G., Klinkhamer, P. G. L., Escobar-Bravo, R., & Leiss, K. A. (2018). Type VI glandular trichome density and their derived volatiles are differently induced by jasmonic acid in developing and fully developed tomato leaves: Implications for thrips resistance. *Plant Science*, *276*, 87–98. <https://doi.org/10.1016/j.plantsci.2018.08.007>
- Chen, M., Yan, T., Shen, Q., Lu, X., Pan, Q., Huang, Y., Tang, Y., Fu, X., Liu, M., & Jiang, W. (2017). GLANDULAR TRICHOME-SPECIFIC WRKY 1 promotes artemisinin biosynthesis in *Artemisia annua*. *New Phytologist*, *214*(1), 304–316.
- Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. <https://doi.org/10.1093/BIOINFORMATICS/BTY560>
- Chen, X., Wang, D. D., Fang, X., Chen, X. Y., & Mao, Y. B. (2019). Plant Specialized Metabolism Regulated by Jasmonate Signaling. *Plant and Cell Physiology*, *60*(12), 2638–2647. <https://doi.org/10.1093/PCP/PCZ161>
- Chen, Y., Su, D., Li, J., Ying, S., Deng, H., He, X., Zhu, Y., Li, Y., Chen, Y., & Pirrello, J. (2020). Overexpression of bHLH95, a basic helix–loop–helix transcription factor family member, impacts trichome formation via regulating gibberellin biosynthesis in tomato. *Journal of Experimental Botany*.

- Chini, A., Ben-Romdhane, W., Hassairi, A., & Aboul-Soud, M. A. M. (2017). Identification of TIFY/JAZ family genes in *Solanum lycopersicum* and their regulation in response to abiotic stresses. *PLoS ONE*, *12*(6), e0177381. <https://doi.org/10.1371/journal.pone.0177381>
- Chiniga Kemparaju, C. (2018). *Identification and characterization of key regulatory components involved in the development of type VI glandular trichomes in Solanum lycopersicum*.
- Chun, J.-I., Kim, S.-M., Jeong, N.-R., Kim, S. H., Jung, C., & Kang, J.-H. (2022). Tomato ARPC1 regulates trichome morphology and density and terpene biosynthesis. *Planta*, *256*(2), 38. <https://doi.org/10.1007/s00425-022-03955-7>
- Chun, J.-I., Kim, S.-M., Kim, H., Cho, J.-Y., Kwon, H.-W., Kim, J.-I., Seo, J.-K., Jung, C., & Kang, J.-H. (2021). SIHair2 Regulates the Initiation and Elongation of Type I Trichomes on Tomato Leaves and Stems. *Plant and Cell Physiology*, *62*(9), 1446–1459. <https://doi.org/10.1093/pcp/pcab090>
- Clement, K., Rees, H., Canver, M. C., Gehrke, J. M., Farouni, R., Hsu, J. Y., Cole, M. A., Liu, D. R., Joung, J. K., Bauer, D. E., & Pinello, L. (2019). CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nature Biotechnology* 2019 *37*:3, *37*(3), 224–226. <https://doi.org/10.1038/s41587-019-0032-3>
- Colby, S. M., Crock, J., Dowdle-Rizzo, B., Lemaux, P. G., & Croteau, R. (1998). Germacrene C synthase from *Lycopersicon esculentum* cv. VFNT Cherry tomato: cDNA isolation, characterization, and bacterial expression of the multiple product sesquiterpene cyclase. *Proceedings of the National Academy of Sciences*, *95*(5), 2216–2221.
- Cortina, C., & Cullianez-Macia, F. A. (2004). Tomato transformation and transgenic plant production. *Plant Cell Tissue and Organ Culture*, *76*(3), 269–275. <https://doi.org/10.1023/B:Ticu.0000009249.14051.77>
- Cui, J., Jiang, N., Zhou, X., Hou, X., Yang, G., Meng, J., & Luan, Y. (2018). Tomato MYB49 enhances resistance to *Phytophthora infestans* and tolerance to water deficit and salt stress. *Planta*, *248*, 1487–1503.
- Dobi, K. C., & Winston, F. (2007). Analysis of Transcriptional Activation at a Distance in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, *27*(15), 5575. <https://doi.org/10.1128/MCB.00459-07>
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15. <https://doi.org/10.1093/BIOINFORMATICS/BTS635>
- Dong, M., Xue, S., Bartholomew, E. S., Zhai, X., Sun, L., Xu, S., Zhang, Y., Yin, S., Ma, W., & Chen, S. (2022). Transcriptomic and functional analysis provides molecular insights into multicellular trichome development. *Plant Physiology*.
- Fahn, A. (2000). Structure and function of secretory cells. *Advances in Botanical Research*, *31*, 37–75. [https://doi.org/10.1016/S0065-2296\(00\)31006-0](https://doi.org/10.1016/S0065-2296(00)31006-0)
- Falara, V., Akhtar, T. A., Nguyen, T. T. H., Spyropoulou, E. A., Bleeker, P. M., Schauvinhold, I., Matsuba, Y., Bonini, M. E., Schillmiller, A. L., Last, R. L., Schuurink, R. C., & Pichersky, E. (2011). The Tomato Terpene Synthase Gene Family. *Plant Physiology*, *157*(2), 770–789. <https://doi.org/10.1104/pp.111.179648>
- Falara, V., Alba, J. M., Kant, M. R., Schuurink, R. C., & Pichersky, E. (2014). Geranylinalool synthases in solanaceae and other angiosperms constitute an ancient branch of diterpene synthases involved in the synthesis of defensive compounds. *Plant Physiology*, *166*(1), 428–441.
- Feng, Z., Bartholomew, E. S., Liu, Z., Cui, Y., Dong, Y., Li, S., Wu, H., Ren, H., & Liu, X. (2021). Glandular trichomes: new focus on horticultural crops. *Horticulture Research*, *8*(1), 158. https://doi.org/10.1038/S41438-021-00592-1/42043349/41438_2021_ARTICLE_592.PDF
- Fernandez-Calvo, P., Chini, A., Fernandez-Barbero, G., Chico, J. M., Gimenez-Ibanez, S., Geerinck, J., Eeckhout, D., Schweizer, F., Godoy, M., Franco-Zorrilla, J. M., Pauwels, L., Witters, E., Puga, M. I., Paz-Ares, J., Goossens, A., Reymond, P., De Jaeger, G., & Solano, R. (2011). The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *Plant Cell*, *23*(2), 701–715. <https://doi.org/10.1105/tpc.110.080788>

- Fernandez-Pozo, N., Rosli, H. G., Martin, G. B., & Mueller, L. A. (2015). The SGN VIGS Tool: User-Friendly Software to Design Virus-Induced Gene Silencing (VIGS) Constructs for Functional Genomics. *Molecular Plant*, 8(3), 486–488. <https://doi.org/https://doi.org/10.1016/j.molp.2014.11.024>
- Fridman, E., Wang, J., Iijima, Y., Froehlich, J. E., Gang, D. R., Ohlrogge, J., & Pichersky, E. (2005). Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. *The Plant Cell*, 17(4), 1252–1267. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1088000/pdf/tpc1701252.pdf>
- Galdon-Armero, J., Arce-Rodriguez, L., Downie, M., Li, J., & Martin, C. (2020). A Scanning Electron Micrograph-based Resource for Identification of Loci Involved in Epidermal Development in Tomato: Elucidation of a New Function for the Mixta-like Transcription Factor in Leaves. *The Plant Cell*, 32(5), 1414 LP – 1433. <https://doi.org/10.1105/tpc.20.00127>
- Galland, M. & Bleeker, P. (2020). *Scaled counts of trichome mRNA-seq from the 20 accessions [Data set]*. <https://doi.org/https://doi.org/10.5281/zenodo.3627091>
- Gao, S. H., Gao, Y. N., Xiong, C., Yu, G., Chang, J., Yang, Q. H., Yang, C. X., & Ye, Z. B. (2017). The tomato B-type cyclin gene, SlCycB2, plays key roles in reproductive organ development, trichome initiation, terpenoids biosynthesis and *Prodenia litura* defense. *Plant Science*, 262, 103–114. <https://doi.org/10.1016/j.plantsci.2017.05.006>
- Gao, Y., Gao, S., Xiong, C., Yu, G., Chang, J., Ye, Z., & Yang, C. (2015). Comprehensive analysis and expression profile of the homeodomain leucine zipper IV transcription factor family in tomato. *Plant Physiology and Biochemistry: PPB*, 96, 141–153. <https://doi.org/10.1016/j.plaphy.2015.07.025>
- Gasperini, D., Chetelat, A., Acosta, I. F., Goossens, J., Pauwels, L., Goossens, A., Dreos, R., Alfonso, E., & Farmer, E. E. (2015). Multilayered Organization of Jasmonate Signalling in the Regulation of Root Growth. *Plos Genetics*, 11(6). https://doi.org/ARTN_e1005300 10.1371/journal.pgen.1005300
- Ge, S. X., Jung, D., Jung, D., & Yao, R. (2020). ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics*, 36(8), 2628–2629. <https://doi.org/10.1093/BIOINFORMATICS/BTZ931>
- Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R. C., & Kant, M. R. (2012). Plant Glandular Trichomes as Targets for Breeding or Engineering of Resistance to Herbivores. *International Journal of Molecular Sciences*, 13(12), 17077–17103. <https://doi.org/10.3390/ijms131217077>
- Gong, S., Ding, Y., Hu, S., Ding, L., Chen, Z., & Zhu, C. (2019). The role of HD-Zip class I transcription factors in plant response to abiotic stresses. *Physiologia Plantarum*, 167(4), 516–525. <https://doi.org/https://doi.org/10.1111/ppl.12965>
- Gong, Z., Luo, Y., Zhang, W., Jian, W., Zhang, L., Gao, X., Hu, X., Yuan, Y., Wu, M., Xu, X., Zheng, X., Wu, G., Li, Z., Li, Z., & Deng, W. (2021). A SIMYB75-centred transcriptional cascade regulates trichome formation and sesquiterpene accumulation in tomato. *Journal of Experimental Botany*, 72(10), 3806–3820. <https://doi.org/10.1093/jxb/erab086>
- Goossens, J., Mertens, J., & Goossens, A. (2017). Role and functioning of bHLH transcription factors in jasmonate signalling. *Journal of Experimental Botany*, 68(6), 1333–1347. <https://doi.org/10.1093/jxb/erw440>
- Gragt van der, M., Therezan, R., & Zocca, P. (2020). *Messenger RNA-seq data from leaves, stem trichomes, bald stems and leaf primordia from different tomato genetic backgrounds [Data set]*. <https://doi.org/https://doi.org/10.5281/zenodo.4326969>
- Grefen, C., & Blatt, M. R. (2012). A 2in1 cloning system enables ratiometric bimolecular fluorescence complementation (rBiFC). *BioTechniques*, 53(5), 311–314. <https://doi.org/10.2144/000113941/ASSET/IMAGES/LARGE/FIGURE2.JPEG>

- Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, *59*(3), 307–321.
- H Kim, M. K., Carroll, W. L., Licht, J., & Bieker, J. (2004). Autoregulation of the N-myc gene is operative in neuroblastoma and involves histone deacetylase 2. *Cancer*, *101*(9), 2106–2115. <https://doi.org/10.1002/CNCR.20626>
- Higo, K., Ugawa, Y., Iwamoto, M., & Korenaga, T. (1999). Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Research*, *27*(1), 297–300. <https://doi.org/10.1093/nar/27.1.297>
- Hong, Y., Liu, Y., Zhang, Y., Jia, L., Yang, X., Zhang, X., Liu, J., & Luan, Y. (2021). Genome-wide characterization of homeobox-leucine zipper gene family in tomato (*Solanum lycopersicum*) and functional analysis of SIHDZ34 (III sub-family member) under salinity stress. *Environmental and Experimental Botany*, *192*, 104652. <https://doi.org/https://doi.org/10.1016/j.envexpbot.2021.104652>
- Hu, J., Chen, G., Yin, W., Cui, B., Yu, X., Lu, Y., & Hu, Z. (2017). Silencing of SIHB2 Improves Drought, Salt Stress Tolerance, and Induces Stress-Related Gene Expression in Tomato. *Journal of Plant Growth Regulation*, *36*(3), 578–589. <https://doi.org/10.1007/s00344-017-9664-z>
- Hu, J., Gao, Y., Zhao, T., Li, J., Yao, M., & Xu, X. (2018). Genome-wide Identification and Expression Pattern Analysis of Zinc-finger Homeodomain Transcription Factors in Tomato under Abiotic Stress. *Journal of the American Society for Horticultural Science J. Amer. Soc. Hort. Sci.*, *143*(1), 14–22. <https://doi.org/10.21273/JASHS04245-17>
- Hua, B., Chang, J., Han, X., Xu, Z., Hu, S., Li, S., Wang, R., Yang, L., Yang, M., Wu, S., Shen, J., Yu, X., & Wu, S. (2022). H and HL synergistically regulate jasmonate-triggered trichome formation in tomato. *Horticulture Research*, *9*. <https://doi.org/10.1093/HR/UHAB080>
- Hua, B., Chang, J., Wu, M., Xu, Z., Zhang, F., Yang, M., Xu, H., Wang, L.-J., Chen, X.-Y., & Wu, S. (2020). Mediation of JA signalling in glandular trichomes by the woolly/SIMYC1 regulatory module improves pest resistance in tomato. *Plant Biotechnology Journal*, *n/a*(n/a). <https://doi.org/10.1111/pbi.13473>
- Hua, B., Chang, J., Xu, Z., Han, X., Xu, M., Yang, M., Yang, C., Ye, Z., & Wu, S. (2021). HOMEODOMAIN PROTEIN8 mediates jasmonate-triggered trichome elongation in tomato. *New Phytologist*, *230*(3), 1063–1077. <https://doi.org/10.1111/NPH.17216>
- J Kortbeek, R. W., Galland, M. D., Muras, A., Therezan, R., Maia, S., Haring, M. A., Schuurink, R. C., Bleeker, P. M., Xu, F., Lindemann, P., Hua, J., Bleeker pmbleeker, P. M., & Rwj, K. (2023). Genetic and physiological requirements for high-level sesquiterpene-production in tomato glandular trichomes. *Frontiers in Plant Science*, *14*, 1139274–1139274. <https://doi.org/10.3389/FPLS.2023.1139274>
- Johnson, H. B. (1975). Plant pubescence: An ecological perspective. *The Botanical Review*, *41*(3), 233–258. <https://doi.org/10.1007/BF02860838>
- Johnson, N. M., & Baucom, R. S. (2024). The double life of trichomes: understanding their dual role in herbivory and herbicide resistance. *Evolution*, *78*(6), 1121–1132. <https://doi.org/10.1093/evolut/qpae048>
- Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., & Tanabe, M. (2021). KEGG: integrating viruses and cellular organisms. *Nucleic Acids Research*, *49*(D1), D545–D551. <https://doi.org/10.1093/NAR/GKAA970>
- Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2023). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research*, *51*(D1), D587–D592. <https://doi.org/10.1093/NAR/GKAC963>
- Kang, J.-H., Campos, M. L., Zemelis-Durfee, S., Al-Haddad, J. M., Jones, A. D., Telewski, F. W., Brandizzi, F., & Howe, G. A. (2016). Molecular cloning of the tomato Hairless gene implicates actin dynamics in trichome-mediated defense and mechanical properties of stem tissue. *Journal of Experimental Botany*, *67*(18), 5313–5324. <https://doi.org/10.1093/jxb/erw292>

- Kang, J.-H., Liu, G., Shi, F., Jones, A. D., Beaudry, R. M., & Howe, G. A. (2010). The tomato odorless-2 mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. *Plant Physiology*, *154*(1), 262–272. <https://doi.org/10.1104/pp.110.160192>
- Kang, J.-H., McRoberts, J., Shi, F., Moreno, J., Jones, D., & Howe, G. A. (2014). The flavonoid biosynthetic enzyme chalcone isomerase modulates terpenoid production in glandular trichomes of tomato. *Plant Physiology*, *113*.233395.
- Kang, J.-H., Shi, F., Jones, A. D., Marks, M. D., & Howe, G. A. (2010). Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. *Journal of Experimental Botany*, *61*(4), 1053–1064. <https://doi.org/10.1093/jxb/erp370>
- Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., & Bresta, P. (2020). Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *Journal of Forestry Research*, *31*(1), 1–12. <https://doi.org/10.1007/s11676-019-01034-4>
- Karimi, M., Inzé, D., & Depicker, A. (2002). GATEWAY™ vectors for Agrobacterium-mediated plant transformation. *Trends in Plant Science*, *7*(5), 193–195.
- Khatun, K., Nath, U. K., Robin, A. H. K., Park, J.-I., Lee, D.-J., Kim, M.-B., Kim, C. K., Lim, K.-B., Nou, I. S., & Chung, M.-Y. (2017a). Genome-wide analysis and expression profiling of zinc finger homeodomain (ZHD) family genes reveal likely roles in organ development and stress responses in tomato. *BMC Genomics*, *18*(1), 1–16.
- Khatun, K., Nath, U. K., Robin, A. H. K., Park, J.-I., Lee, D.-J., Kim, M.-B., Kim, C. K., Lim, K.-B., Nou, I. S., & Chung, M.-Y. (2017b). Genome-wide analysis and expression profiling of zinc finger homeodomain (ZHD) family genes reveal likely roles in organ development and stress responses in tomato. *BMC Genomics*, *18*(1), 1–16.
- Koch, K., Bhushan, B., & Barthlott, W. (2009). Multifunctional surface structures of plants: An inspiration for biomimetics. *Progress in Materials Science*, *54*(2), 137–178. <https://doi.org/https://doi.org/10.1016/j.pmatsci.2008.07.003>
- Kortbeek, R. W. J. (2022). *Defence from the wild: specialised metabolism in tomato glandular trichomes* [Thesis].
- Kortbeek, R. W. J., Galland, M. D., Muras, A., van der Kloet, F. M., André, B., Heilijgers, M., van Hijum, S. A. F. T., Haring, M. A., Schuurink, R. C., & Bleeker, P. M. (2021). Natural variation in wild tomato trichomes; selecting metabolites that contribute to insect resistance using a random forest approach. *BMC Plant Biology* *2021* *21:1*, *21*(1), 1–19. <https://doi.org/10.1186/S12870-021-03070-X>
- Kortbeek, R. W., Xu, J., Ramirez, A., Spyropoulou, E., Diergaarde, P., Otten-Bruggeman, I., de Both, M., Nagel, R., Schmidt, A., Schuurink, R. C., & Bleeker, P. M. (2016). Engineering of Tomato Glandular Trichomes for the Production of Specialized Metabolites. *Methods Enzymol*, *576*, 305–331. <https://doi.org/10.1016/bs.mie.2016.02.014>
- Landi, M., Tattini, M., & Gould, K. S. (2015). Multiple functional roles of anthocyanins in plant-environment interactions. *Environmental and Experimental Botany*, *119*, 4–17. <https://doi.org/https://doi.org/10.1016/j.envexpbot.2015.05.012>
- Lange, B. M. (2015). The Evolution of Plant Secretory Structures and Emergence of Terpenoid Chemical Diversity. *Annual Review of Plant Biology*, *66*(1), 139–159. <https://doi.org/10.1146/annurev-arplant-043014-114639>
- Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y., van de Peer, Y., Rouzé, P., & Rombauts, S. (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Research*, *30*(1), 325–327. <https://doi.org/10.1093/nar/30.1.325>
- Levin, D. A. (1973). The role of trichomes in plant defense. *The Quarterly Review of Biology*, *48*(1, Part 1), 3–15.
- Li, H. (2021). New strategies to improve minimap2 alignment accuracy. *Bioinformatics*, *37*(23), 4572–4574. <https://doi.org/10.1093/BIOINFORMATICS/BTAB705>

- Li, J., Luan, Y., & Jin, H. (2012). The tomato SIWRKY gene plays an important role in the regulation of defense responses in tobacco. *Biochemical and Biophysical Research Communications*, 427(3), 671–676. <https://doi.org/10.1016/j.bbrc.2012.09.120>
- Li, L., Zhao, Y., McCaig, B. C., Wingerd, B. A., Wang, J., Whalon, M. E., Pichersky, E., & Howe, G. A. (2004). The tomato homolog of Coronatine-insensitive1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell*, 16(3), 783. <https://doi.org/10.1105/tpc.cor650>
- Li, Q., Cao, C., Zhang, C., Zheng, S., Wang, Z., Wang, L., & Ren, Z. (2015). The identification of *Cucumis sativus* Glabrous 1 (CsGL1) required for the formation of trichomes uncovers a novel function for the homeodomain-leucine zipper I gene. *Journal of Experimental Botany*, 66(9), 2515–2526.
- Li, R., Wang, X., Zhang, S., Liu, X., Zhou, Z., Liu, Z., Wang, K., Tian, Y., Wang, H., Zhang, Y., & Cui, X. (2021). Two zinc-finger proteins control the initiation and elongation of long stalk trichomes in tomato. *Journal of Genetics and Genomics*, 48(12), 1057–1069. <https://doi.org/10.1016/J.JGG.2021.09.001>
- Li, Y., Chen, Y., Zhou, L., You, S., Deng, H., Chen, Y., Alseekh, S., Yuan, Y., Fu, R., & Zhang, Z. (2020). MicroTom metabolic network: rewiring tomato metabolic regulatory network throughout the growth cycle. *Molecular Plant*, 13(8), 1203–1218.
- Li, Y., Zhang, X., Jiang, J., Zhao, T., Xu, X., Yang, H., & Li, J. (2022). Virus-induced gene silencing of SIPYL4 decreases the drought tolerance of tomato. *Horticultural Plant Journal*, 8(3), 361–368. <https://doi.org/10.1016/J.HPJ.2021.06.005>
- Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7), 923–930. <https://doi.org/10.1093/BIOINFORMATICS/BTT656>
- Liu, Y., Schiff, M., & Dinesh-Kumar, S. P. (2002). Virus-induced gene silencing in tomato. *The Plant Journal*, 31(6), 777–786.
- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 1–21. <https://doi.org/10.1186/S13059-014-0550-8/FIGURES/9>
- Lucini, T., Faria, M. V., Rohde, C., Resende, J. T. V., & de Oliveira, J. R. F. (2015). Acylsugar and the role of trichomes in tomato genotypes resistance to *Tetranychus urticae*. *Arthropod-Plant Interactions*, 9, 45–53.
- Luckwill, L. C. (1943). The genus *Lycopersicon*. *Aberdeen Univ. Studies*, 120.
- Madeira, F., Pearce, M., Tivey, A. R. N., Basutkar, P., Lee, J., Edbali, O., Madhusoodanan, N., Kolesnikov, A., & Lopez, R. (2022). Search and sequence analysis tools services from EMBL-EBI in 2022. *Nucleic Acids Research*, 50(W1), W276–W279. <https://doi.org/10.1093/NAR/GKAC240>
- Major, I. T., Yoshida, Y., Campos, M. L., Kapali, G., Xin, X.-F., Sugimoto, K., de Oliveira Ferreira, D., He, S. Y., & Howe, G. A. (2017). Regulation of growth-defense balance by the JASMONATE ZIM-DOMAIN (JAZ)-MYC transcriptional module. *New Phytologist*, 215(4), 1533–1547. <https://doi.org/10.1111/nph.14638>
- Mandal, S., Ji, W., & McKnight, T. D. (2020). Candidate Gene Networks for Acylsugar Metabolism and Plant Defense in Wild Tomato *Solanum pennellii*. *The Plant Cell*, 32(1), 81 LP – 99. <https://doi.org/10.1105/tpc.19.00552>
- Matsumura, M., Nomoto, M., Itaya, T., Aratani, Y., Iwamoto, M., Matsuura, T., Hayashi, Y., Mori, T., Skelly, M. J., Yamamoto, Y. Y., Kinoshita, T., Mori, I. C., Suzuki, T., Betsuyaku, S., Spoel, S. H., Toyota, M., & Tada, Y. (2022). Mechanosensory trichome cells evoke a mechanical stimuli-induced immune response in *Arabidopsis thaliana*. *Nature Communications*, 13(1), 1216. <https://doi.org/10.1038/s41467-022-28813-8>
- McDowell, E. T., Kapteyn, J., Schmidt, A., Li, C., Kang, J.-H., Descour, A., Shi, F., Larson, M., Schillmiller, A., An, L., Jones, A. D., Pichersky, E., Soderlund, C. A., & Gang, D. R. (2011). Comparative Functional Genomic Analysis of *Solanum* Glandular Trichome Types. *Plant Physiology*, 155(1), 524–539. <https://doi.org/10.1104/pp.110.167114>

- Melotto, M., Mecey, C., Niu, Y., Chung, H. S., Katsir, L., Yao, J., Zeng, W., Thines, B., Staswick, P., Browse, J., Howe, G. A., & He, S. Y. (2008). A critical role of two positively charged amino acids in the Jas motif of Arabidopsis JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. *The Plant Journal*, *55*(6), 979–988. <https://doi.org/10.1111/J.1365-313X.2008.03566.X>
- Moore, R. M., Harrison, A. O., McAllister, S. M., Polson, S. W., & Wommack, K. E. (2020). Iroki: automatic customization and visualization of phylogenetic trees. *PeerJ*, *8*, e8584.
- Naalden, D., Dermauw, W., Ilias, A., Baggerman, G., Mastop, M., Dangol, S., Gaertner, N., Roseboom, W., Kwaaitaal, M., Kramer, G., Burg, H. A. van den, Vontas, J., Leeuwen, T. Van, Kant, M. R., & Schuurink, R. C. (2023). Whitefly effector G4 interacts with tomato proteins of which MIPDB141 affects whitefly performance. *BioRxiv*, 2023.03.11.532171. <https://doi.org/10.1101/2023.03.11.532171>
- Nadakuduti, S. S., Pollard, M., Kosma, D. K., Allen Jr., C., Ohlrogge, J. B., & Barry, C. S. (2012). Pleiotropic Phenotypes of the sticky peel Mutant Provide New Insight into the Role of CUTIN DEFICIENT2 in Epidermal Cell Function in Tomato. *Plant Physiology*, *159*(3), 945–960. <https://doi.org/10.1104/pp.112.198374>
- Nakashima, T., Wada, H., Morita, S., Erra-Balsells, R., Hiraoka, K., & Nonami, H. (2016). Single-cell metabolite profiling of stalk and glandular cells of intact trichomes with internal electrode capillary pressure probe electrospray ionization mass spectrometry. *Analytical Chemistry*, *88*(6), 3049–3057. <https://pubs.acs.org/doi/pdfplus/10.1021/acs.analchem.5b03366>
- Napoli, C., Lemieux, C., & Jorgensen, R. (1990). Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes in trans. *The Plant Cell*, *2*(4), 279–289. <https://doi.org/10.1105/TPC.2.4.279>
- Natale, R., Coppola, M., D’Agostino, N., Zhang, Y., Fernie, A. R., Castaldi, V., & Rao, R. (2023). In silico and in vitro approaches allow the identification of the Prosystemin molecular network. *Computational and Structural Biotechnology Journal*, *21*, 212–223. <https://doi.org/https://doi.org/10.1016/j.csbj.2022.12.006>
- Ouwerkerk, P. B. F., & Meijer, A. H. (2011). Yeast one-hybrid screens for detection of transcription factor DNA interactions. *Methods in Molecular Biology (Clifton, N.J.)*, *678*, 211–227. https://doi.org/10.1007/978-1-60761-682-5_16/COVER
- Pan, J., Zhang, L., Chen, G., Wen, H., Chen, Y., Du, H., Zhao, J., He, H., Lian, H., & Chen, H. (2021). Study of micro-trichome (mict) reveals novel connections between transcriptional regulation of multicellular trichome development and specific metabolism in cucumber. *Horticulture Research*, *8*.
- Panda, S., Busatto, N., Hussain, K., & Kamble, A. (2019). Piriformospora indica-primed transcriptional reprogramming induces defense response against early blight in tomato. *Scientia Horticulturae*, *255*, 209–219.
- Panda, S., Jozwiak, A., Sonawane, P. D., Szymanski, J., Kazachkova, Y., Vainer, A., Vasuki, H., Almekias-Siegl, E., Dikaya, V., & Bocobza, S. (2021). Steroidal Alkaloids Defense Metabolism and Plant Growth are Modulated by the Joint Action of Gibberellin and Jasmonate Signaling. *New Phytologist*.
- Panda, S., Jozwiak, A., Sonawane, P. D., Szymanski, J., Kazachkova, Y., Vainer, A., Vasuki Kilambi, H., Almekias-Siegl, E., Dikaya, V., Bocobza, S., Shohat, H., Meir, S., Wizler, G., Giri, A. P., Schuurink, R., Weiss, D., Yasuor, H., Kamble, A., & Aharoni, A. (2022). Steroidal alkaloids defence metabolism and plant growth are modulated by the joint action of gibberellin and jasmonate signalling. *New Phytologist*, *233*(3), 1220–1237. <https://doi.org/10.1111/NPH.17845>
- Pauwels, L., Barbero, G. F., Geerinck, J., Tilleman, S., Grunewald, W., Pérez, A. C., Chico, J. M., Bossche, R. Vanden, Sewell, J., & Gil, E. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature*, *464*(7289), 788. <https://www.nature.com/articles/nature08854.pdf>
- Payne, W. W. (1978). A glossary of plant hair terminology. *Brittonia*, *30*(2), 239–255.

- Pedregosa, F., Michel, V., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Vanderplas, J., Cournapeau, D., Pedregosa, F., Varoquaux, G., Gramfort, A., Thirion, B., Grisel, O., Dubourg, V., Passos, A., Brucher, M., Perrot, M., & Duchesnay, É. (2011). Scikit-learn: Machine Learning in Python. *Journal of Machine Learning Research*, *12*, 2825–2830. <http://scikit-learn.sourceforge.net>.
- Rajeevkumar, S., Anunanthini, P., & Sathishkumar, R. (2015). Epigenetic silencing in transgenic plants. *Frontiers in Plant Science*, *6*(September), 152056. <https://doi.org/10.3389/FPLS.2015.00693/BIBTEX>
- Rakha, M., Bouba, N., Ramasamy, S., Regnard, J.-L., & Hanson, P. (2017). Evaluation of wild tomato accessions (*Solanum* spp.) for resistance to two-spotted spider mite (*Tetranychus urticae* Koch) based on trichome type and acylsugar content. *Genetic Resources and Crop Evolution*, *64*, 1011–1022.
- Reback, J., McKinney, W., Van Den Bossche, J., Augspurger, T., Cloud, P., Klein, A., Hawkins, S., Roeschke, M., Tratner, J., & She, C. (2020). pandas-dev/pandas: Pandas 1.0. 5. *Zenodo*.
- Reece-Hoyes, J. S., & Marian Walhout, A. J. (2012). Yeast one-hybrid assays: A historical and technical perspective. *Methods*, *57*(4), 441–447. <https://doi.org/10.1016/J.YMETH.2012.07.027>
- Robinson, J. T., Thorvaldsdottir, H., Turner, D., & Mesirov, J. P. (2023). igv.js: an embeddable JavaScript implementation of the Integrative Genomics Viewer (IGV). *Bioinformatics*, *39*(1). <https://doi.org/10.1093/BIOINFORMATICS/BTAC830>
- Roylawar, P., Panda, S., & Kamble, A. (2015). Comparative analysis of BABA and Piriformospora indica mediated priming of defence-related genes in tomato against early blight. *Physiological and Molecular Plant Pathology*, *91*, 88–95.
- Schaart, J. G., Dubos, C., De La Fuente, I. R., van Houwelingen, A. M. M. L., de Vos, R. C. H., Jonker, H. H., Xu, W. J., Routaboul, J. M., Lepiniec, L., & Bovy, A. G. (2013). Identification and characterization of MYB-bHLH-WD40 regulatory complexes controlling proanthocyanidin biosynthesis in strawberry (*Fragaria x ananassa*) fruits. *New Phytologist*, *197*(2), 454–467. <https://doi.org/10.1111/nph.12017>
- Schillmiller, A. L., Last, R. L., & Pichersky, E. (2008). Harnessing plant trichome biochemistry for the production of useful compounds. *The Plant Journal*, *54*(4), 702–711.
- Schillmiller, A. L., Miner, D. P., Larson, M., McDowell, E., Gang, D. R., Wilkerson, C., & Last, R. L. (2010a). Studies of a biochemical factory: tomato trichome deep EST sequencing and proteomics. *Plant Physiology*, *110*.157214.
- Schillmiller, A. L., Miner, D. P., Larson, M., McDowell, E., Gang, D. R., Wilkerson, C., & Last, R. L. (2010b). Studies of a biochemical factory: tomato trichome deep expressed sequence tag sequencing and proteomics. *Plant Physiology*, *153*(3), 1212–1223. <https://doi.org/10.1104/pp.110.157214>
- Schillmiller, A. L., Chauvinhold, I., Larson, M., Xu, R., Charbonneau, A. L., Schmidt, A., Wilkerson, C., Last, R. L., & Pichersky, E. (2009). Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(26), 10865–10870. <https://doi.org/10.1073/pnas.0904113106>
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods* *2012* 9:7, *9*(7), 676–682. <https://doi.org/10.1038/nmeth.2019>
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
- Schuurink, R., & Tissier, A. (2020). Glandular trichomes: micro-organs with model status? *New Phytologist*, *225*(6), 2251–2266. [10.1111/nph.16283](https://doi.org/10.1111/nph.16283)
- Shi, Y., Pang, X., Liu, W., Wang, R., Su, D., Gao, Y., Wu, M., Deng, W., Liu, Y., & Li, Z. (2021). SIZHD17 is involved in the control of chlorophyll and carotenoid metabolism in tomato fruit. *Horticulture*

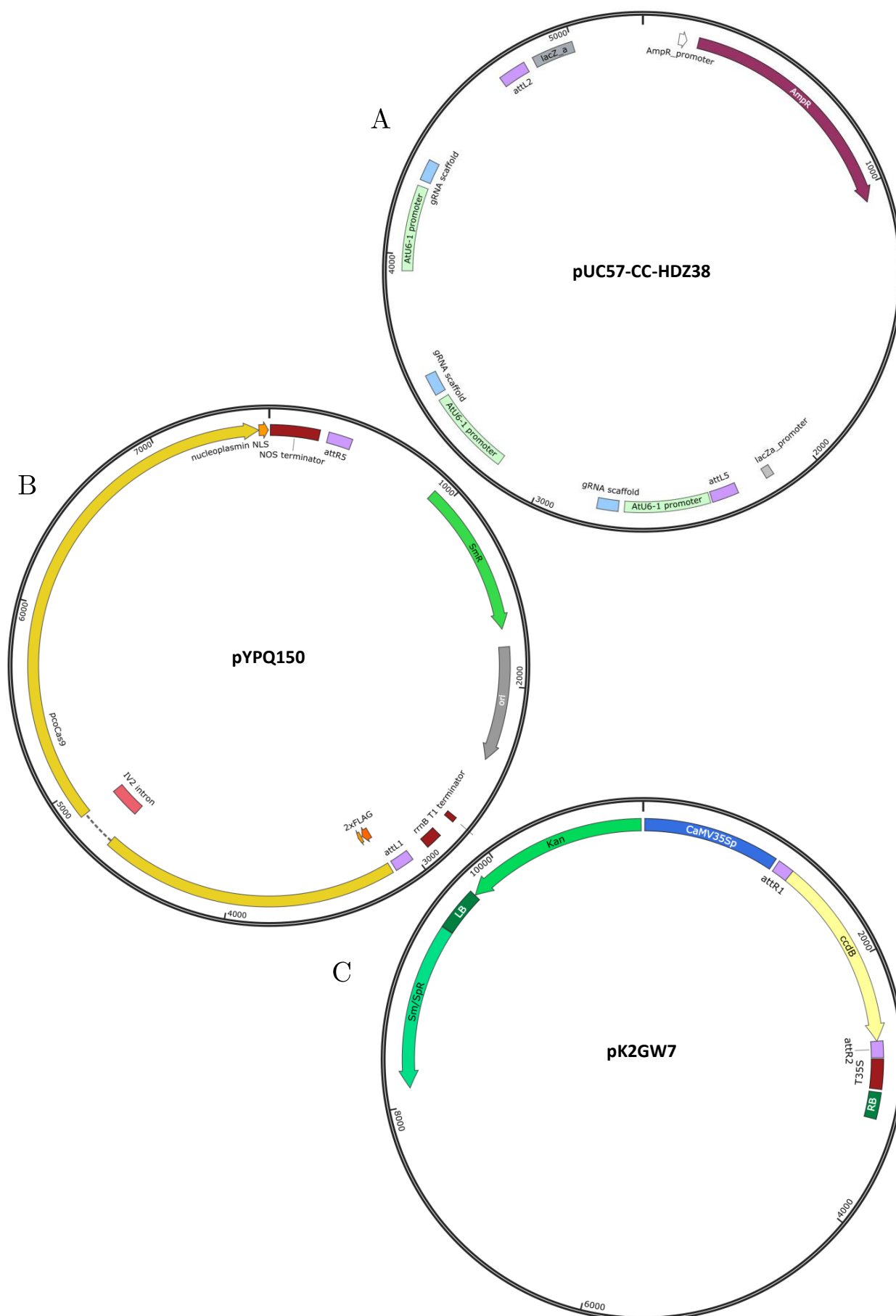
- Research*, 8(1), 259. https://doi.org/10.1038/S41438-021-00696-8/42042263/41438_2021_ARTICLE_696.PDF
- Silva, K. F. A. S., Michereff-Filho, M., Fonseca, M. E. N., Silva-Filho, J. G., Texeira, A. C. A., Moita, A. W., Torres, J. B., Fernández-Muñoz, R., & Boiteux, L. S. (2014). Resistance to *B. emisia tabaci* biotype B of *Solanum pimpinellifolium* is associated with higher densities of type IV glandular trichomes and acylsugar accumulation. *Entomologia Experimentalis et Applicata*, 151(3), 218–230.
- Simmons, A. T., & Gurr, G. M. (2005). Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. *Agricultural and Forest Entomology*, 7(4), 265–276.
- Spiegel, J., Arnone, J. T., & Grieco, F. (2021). Transcription at a Distance in the Budding Yeast *Saccharomyces cerevisiae*. *Applied Microbiology 2021, Vol. 1, Pages 142-149*, 1(1), 142–149. <https://doi.org/10.3390/APPLMICROBIOL1010011>
- Spyropoulou, E. (2012). *Transcription factors regulating terpene synthases in tomato trichomes* [University of Amsterdam]. <https://hdl.handle.net/11245/1.376175>
- Spyropoulou, E. A., Haring, M. A., & Schuurink, R. C. (2014a). Expression of Terpenoids 1, a glandular trichome-specific transcription factor from tomato that activates the terpene synthase 5 promoter. *Plant Molecular Biology*, 84(3), 345–357. <https://link.springer.com/content/pdf/10.1007%2Fs11103-013-0142-0.pdf>
- Spyropoulou, E. A., Haring, M. A., & Schuurink, R. C. (2014b). RNA sequencing on *Solanum lycopersicum* trichomes identifies transcription factors that activate terpene synthase promoters. *BMC Genomics*, 15(1), 402. <https://doi.org/Artn40210.1186/1471-2164-15-402>
- Stemmer, M., Thumberger, T., del Sol Keyer, M., Wittbrodt, J., & Mateo, J. L. (2015). CCTop: An Intuitive, Flexible and Reliable CRISPR/Cas9 Target Prediction Tool. *PLOS ONE*, 10(4), e0124633. <https://doi.org/10.1371/journal.pone.0124633>
- Sun, C., Wei, J., Gu, X., Wu, M., Li, M., Liu, Y., An, N., Wu, K., Wu, S., Wu, J., Xu, M., Wu, J., Wang, Y., Chao, D., Zhang, Y., & Wu, S. (2024). Different multicellular trichome types coordinate herbivore mechanosensing and defense in tomato. *The Plant Cell*, koae269. <https://doi.org/10.1093/plcell/koae269>
- Sunilkumar, G., Mohr, L., Lopata-Finch, E., ... C. E.-P. molecular, & 2002, undefined. (2002). Developmental and tissue-specific expression of CaMV 35S promoter in cotton as revealed by GFP. *SpringerG Sunilkumar, LA Mohr, E Lopata-Finch, C Emani, KS RathorePlant Molecular Biology, 2002•Springer, 50(3)*, 463–479. <https://doi.org/10.1023/A:1019832123444>
- Swinnen, G., De Meyer, M., Pollier, J., Molina-Hidalgo, F. J., Ceulemans, E., De Clercq, R., Bossche, R. Vanden, Fernández-Calvo, P., Ron, M., Pauwels, L., & Goossens, A. (2020). Constitutive Steroidal Glycoalkaloid Biosynthesis in Tomato is Regulated by the Clade IIIe Basic Helix-Loop-Helix Transcription Factors MYC1 and MYC2. *BioRxiv*, 2020.01.27.921833. <https://doi.org/10.1101/2020.01.27.921833>
- Swinnen, G., De Meyer, M., Pollier, J., Molina-Hidalgo, F. J., Ceulemans, E., Venegas-Molina, J., De Milde, L., Fernández-Calvo, P., Ron, M., Pauwels, L., & Goossens, A. (2022a). The basic helix–loop–helix transcription factors MYC1 and MYC2 have a dual role in the regulation of constitutive and stress-inducible specialized metabolism in tomato. *New Phytologist*, n/a(n/a). <https://doi.org/https://doi.org/10.1111/nph.18379>
- Swinnen, G., De Meyer, M., Pollier, J., Molina-Hidalgo, F. J., Ceulemans, E., Venegas-Molina, J., De Milde, L., Fernández-Calvo, P., Ron, M., Pauwels, L., & Goossens, A. (2022b). The basic helix–loop–helix transcription factors MYC1 and MYC2 have a dual role in the regulation of constitutive and stress-inducible specialized metabolism in tomato. *New Phytologist*, 236(3), 911–928. <https://doi.org/10.1111/NPH.18379>
- Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, 51(D1), D638–D646. <https://doi.org/10.1093/NAR/GKAC1000>

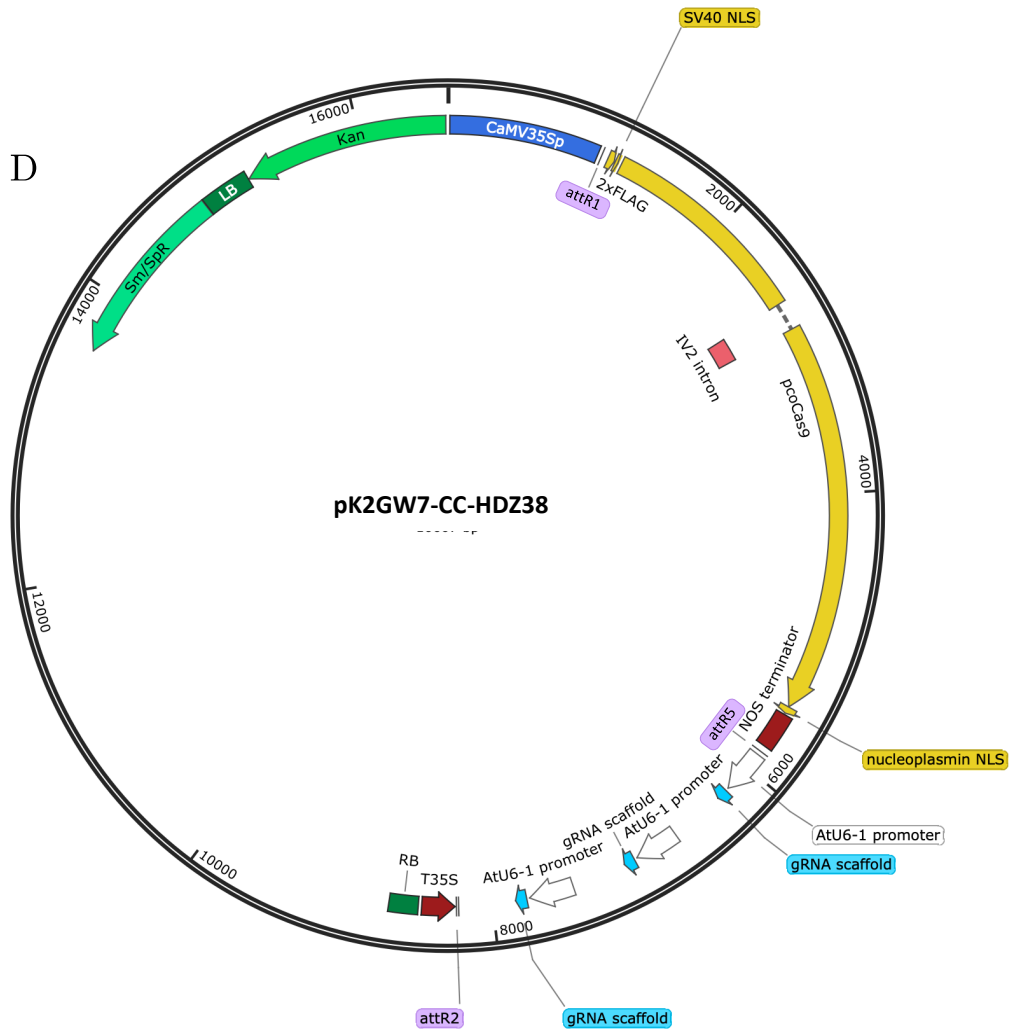
- Tan, Q. K. G., & Irish, V. F. (2006). The Arabidopsis Zinc Finger-Homeodomain Genes Encode Proteins with Unique Biochemical Properties That Are Coordinately Expressed during Floral Development. *Plant Physiology*, *140*(3), 1095–1108. <https://doi.org/10.1104/PP.105.070565>
- Therezan, R., Kortbeek, R., Vendemiatti, E., Legarrea, S., de Alencar, S. M., Schuurink, R., Bleeker, P., P Peres, L. E., & Estáquio Pereira Peres, L. (2020). Genetic factors outside the metabolic cluster for plastid-derived sesquiterpenes are required to pursue arthropod-resistant tomatoes. *BioRxiv*, 2020.02.21.960112. <https://doi.org/10.1101/2020.02.21.960112>
- Therezan, R., Kortbeek, R., Vendemiatti, E., Legarrea, S., de Alencar, S. M., Schuurink, R. C., Bleeker, P., & Peres, L. E. P. (2021). Introgression of the sesquiterpene biosynthesis from *Solanum habrochaites* to cultivated tomato offers insights into trichome morphology and arthropod resistance. *Planta*, *254*(1). <https://doi.org/10.1007/s00425-021-03651-y>
- Tian, D., Tooker, J., Peiffer, M., Chung, S. H., & Felton, G. W. (2012). Role of trichomes in defense against herbivores: comparison of herbivore response to woolly and hairless trichome mutants in tomato (*Solanum lycopersicum*). *Planta*, *236*(4), 1053–1066. <https://link.springer.com/content/pdf/10.1007%2Fs00425-012-1651-9.pdf>
- Tian, T., Liu, Y., Yan, H., You, Q., Yi, X., Du, Z., Xu, W., & Su, Z. (2017). agriGO v2.0: a GO analysis toolkit for the agricultural community, 2017 update. *Nucleic Acids Research*, *45*(W1), W122–W129. <https://doi.org/10.1093/NAR/GKX382>
- Tissier, A. (2012). Glandular trichomes: what comes after expressed sequence tags? *Plant Journal*, *70*(1), 51–68. <https://doi.org/10.1111/j.1365-313X.2012.04913.x>
- Tissier, A. (2018). Plant secretory structures: more than just reaction bags. *Current Opinion in Biotechnology*, *49*, 73–79. <https://doi.org/10.1016/j.copbio.2017.08.003>
- Tissier, A., Morgan, J. A., & Dudareva, N. (2017a). Plant Volatiles: Going “In” but not “Out” of Trichome Cavities. *Trends in Plant Science*, *22*(11), 930–938. <https://doi.org/10.1016/j.tplants.2017.09.001>
- Tissier, A., Morgan, J. A., & Dudareva, N. (2017b). Plant Volatiles: Going “In” but not “Out” of Trichome Cavities. *Trends in Plant Science*, *22*(11), 930–938. <https://doi.org/10.1016/j.tplants.2017.09.001>
- Tomato Genome Consortium, xx. (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, *485*(7400), 635.
- Valentine, T., Shaw, J., Blok, V. C., Phillips, M. S., Oparka, K. J., & Lacomme, C. (2004). Efficient virus-induced gene silencing in roots using a modified tobacco rattle virus vector. *Plant Physiology*, *136*(4), 3999–4009.
- van Engelen, F. A., Molthoff, J. W., Conner, A. J., Nap, J.-P., Pereira, A., & Stiekema, W. J. (1995). pBINPLUS: an improved plant transformation vector based on pBIN19. *Transgenic Research*, *4*(4), 288–290.
- van Schie, C. C. N., Haring, M. A., & Schuurink, R. C. (2007). Tomato linalool synthase is induced in trichomes by jasmonic acid. *Plant Molecular Biology*, *64*, 251–263.
- Vendemiatti, E., Zsögön, A., Silva, G. F. F. e, de Jesus, F. A., Cutri, L., Figueiredo, C. R. F., Tanaka, F. A. O., Nogueira, F. T. S., & Peres, L. E. P. (2017). Loss of type-IV glandular trichomes is a heterochronic trait in tomato and can be reverted by promoting juvenility. *Plant Science*, *259*, 35–47. <https://doi.org/https://doi.org/10.1016/j.plantsci.2017.03.006>
- Vermeij, G. J. (2015). Plants that lead: do some surface features direct enemy traffic on leaves and stems? *Biological Journal of the Linnean Society*, *116*(2), 288–294.
- Veronico, P., Rosso, L. C., Melillo, M. T., Fanelli, E., De Luca, F., Ciancio, A., Colagiero, M., & Pentimone, I. (2022). Water stress differentially modulates the expression of tomato cell wall metabolism-related genes in meloidogyne incognita feeding sites. *Frontiers in Plant Science*, *13*, 817185.
- Verweij, W., Spelt, C., di Sansebastiano, G.-P., Vermeer, J., Reale, L., Ferranti, F., Koes, R., & Quattrocchio, F. (2008). An H⁺ P-ATPase on the tonoplast determines vacuolar pH and flower colour. *Nature Cell Biology*, *10*(12), 1456–1462. <https://doi.org/10.1038/ncb1805>
- Voinnet, O., Rivas, S., Mestre, P., & Baulcombe, D. (2003). Retracted: An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato

- bushy stunt virus. *The Plant Journal*, 33(5), 949–956. <https://doi.org/10.1046/j.1365-313x.2003.01676.x>.
- Wagner, G. J., Wang, E., & Sheperd, R. W. (2004). New Approaches for Studying and Exploiting an Old Protuberance, the Plant Trichome. *Annals of Botany*, 93(1), 3–11. <https://doi.org/10.1093/aob/mch011>
- Wang, Q., Xue, D., Chen, X., Li, J., & Wang, A. (2017). Bioinformatics and functional analysis of WRKY transcription factors in interaction between tomato and *Cladosporium fulvum*. *Journal of Henan Agricultural Sciences*, 46(11), 74–79.
- Wang, W., Wu, P., Li, Y., & Hou, X. L. (2016). Genome-wide analysis and expression patterns of ZF-HD transcription factors under different developmental tissues and abiotic stresses in Chinese cabbage. *Molecular Genetics and Genomics*, 291(3), 1451–1464. <https://doi.org/10.1007/S00438-015-1136-1/METRICS>
- Wang, Z., Yan, X., Zhang, H., Meng, Y., Pan, Y., & Cui, H. (2021). NtCycB2 negatively regulates tobacco glandular trichome formation, exudate accumulation, and aphid resistance. *Plant Molecular Biology*. <https://doi.org/10.1007/s11103-021-01222-z>
- Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M., & Barton, G. J. (2009). Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics*, 25(9), 1189–1191. <https://doi.org/10.1093/BIOINFORMATICS/BTP033>
- Weinhold, A., & Baldwin, I. T. (2011). Trichome-derived O-acyl sugars are a first meal for caterpillars that tags them for predation. *Proceedings of the National Academy of Sciences*, 108(19), 7855–7859.
- Werker, E. (2000). *Trichome diversity and development*.
- Wu, M., Bian, X., Hu, S., Huang, B., Shen, J., Du, Y., Wang, Y., Xu, M., Xu, H., Yang, M., & Wu, S. (2024). A gradient of the HD-Zip regulator Woolly regulates multicellular trichome morphogenesis in tomato. *The Plant Cell*, 36(6), 2375–2392. <https://doi.org/10.1093/plcell/koae077>
- Wu, M., Chang, J., Han, X., Shen, J., Yang, L., Hu, S., Huang, B.-B., Xu, H., Xu, M., Wu, S., Li, P., Hua, B., Yang, M., Yang, Z., & Wu, S. (2023). A HD-ZIP transcription factor specifies fates of multicellular trichomes via dosage-dependent mechanisms in tomato. *Developmental Cell*, 58(4), 278–288.e5. <https://doi.org/10.1016/j.devcel.2023.01.009>
- Wu, S., Hu, C., Zhu, C., Fan, Y., Zhou, J., Xia, X., Shi, K., Zhou, Y., Foyer, C. H., & Yu, J. (2024). The MYC2-PUB22-JAZ4 module plays a crucial role in jasmonate signaling in tomato. *Molecular Plant*, 17(4), 598–613. <https://doi.org/10.1016/j.molp.2024.02.006>
- Xie, Q., Gao, Y., Li, J., Yang, Q., Qu, X., Li, H., Zhang, J., Wang, T., Ye, Z., & Yang, C. (2020). The HD-Zip IV transcription factor SIHDZIV8 controls multicellular trichome morphology by regulating the expression of Hairless-2. *Journal of Experimental Botany*. <https://doi.org/10.1093/jxb/eraa428>
- Xie, Q., Xiong, C., Yang, Q., Zheng, F., Larkin, R. M., Zhang, J., Wang, T., Zhang, Y., Ouyang, B., Lu, Y., Ye, J., Ye, Z., & Yang, C. (2022). A novel regulatory complex mediated by Lanata (Ln) controls multicellular trichome formation in tomato. *New Phytologist*. <https://doi.org/10.1111/NPH.18492>
- Xu, J. (2023). *Transcriptional regulation of volatile terpene biosynthesis in tomato type VI trichomes*.
- Xu, J., van Herwijnen, Z. O., Drager, D. B., Sui, C., Haring, M. A., & Schuurink, R. C. (2018). SIMYC1 Regulates Type VI Glandular Trichome Formation and Terpene Biosynthesis in Tomato Glandular Cells. *Plant Cell*. <https://doi.org/10.1105/tpc.18.00571>
- Yan, L., Zhai, Q., Wei, J., Li, S., Wang, B., Huang, T., Du, M., Sun, J., Kang, L., Li, C. B., & Li, C. (2013). Role of tomato lipoxygenase D in wound-induced jasmonate biosynthesis and plant immunity to insect herbivores. *Plos Genetics*, 9(12), e1003964. <https://doi.org/10.1371/journal.pgen.1003964>
- Yang, C., Li, H., Zhang, J., Luo, Z., Gong, P., Zhang, C., Li, J., Wang, T., Zhang, Y., Lu, Y., & Ye, Z. (2011). A regulatory gene induces trichome formation and embryo lethality in tomato. *Proceedings of the National Academy of Sciences*, 108(29), 11836–11841. <https://doi.org/10.1073/pnas.1100532108>

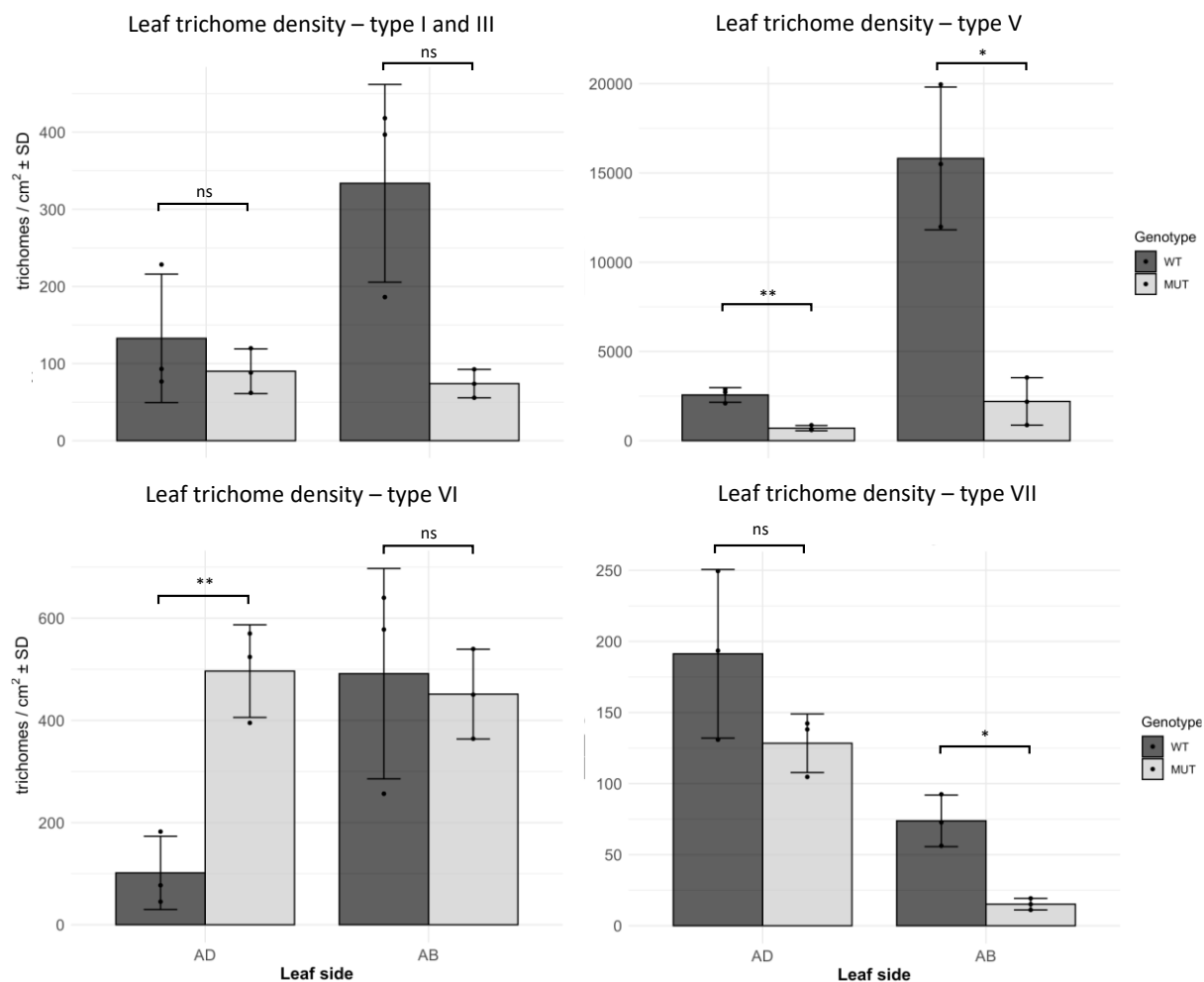
- Yang, C., Li, H., Zhang, J., Wang, T., & Ye, Z. (2011). Fine-mapping of the woolly gene controlling multicellular trichome formation and embryonic development in tomato. *Theoretical and Applied Genetics*, *123*(4), 625–633. <https://doi.org/10.1007/s00122-011-1612-x>
- Yang, C., Marillonnet, S., & Tissier, A. (2021). The Scarecrow-Like Transcription Factor SISCL3 Regulates Volatile Terpene Biosynthesis and Glandular Trichome Size in Tomato (*Solanum lycopersicum*). *The Plant Journal: For Cell and Molecular Biology*.
- Ying, S., Su, M., Wu, Y., Zhou, L., Fu, R., Li, Y., Guo, H., Luo, J., Wang, S., & Zhang, Y. (2020). Trichome regulator SIMIXTA-like directly manipulates primary metabolism in tomato fruit. *Plant Biotechnology Journal*, *18*(2), 354–363. <https://doi.org/10.1111/pbi.13202>
- Yoshida, Y., Sano, R., Wada, T., Takabayashi, J., & Okada, K. (2009). Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in Arabidopsis. *Development*, *136*(6), 1039–1048. <https://doi.org/10.1242/dev.030585>
- Yu, X., Chen, G., Tang, B., Zhang, J., Zhou, S., & Hu, Z. (2018). The Jasmonate ZIM-domain protein gene SIJAZ2 regulates plant morphology and accelerates flower initiation in *Solanum lycopersicum* plants. *Plant Science*, *267*, 65–73. <https://doi.org/https://doi.org/10.1016/j.plantsci.2017.11.008>
- Yuan, Y., Xu, X., Luo, Y., Gong, Z., Hu, X., Wu, M., Liu, Y., Yan, F., Zhang, X., & Zhang, W. (2021). R2R3 MYB-dependent auxin signalling regulates trichome formation, and increased trichome density confers spider mite tolerance on tomato. *Plant Biotechnology Journal*, *19*(1), 138–152.
- Zhang, F., Yao, J., Ke, J., Zhang, L., Lam, V. Q., Xin, X. F., Zhou, X. E., Chen, J., Brunzelle, J., Griffin, P. R., Zhou, M., Xu, H. E., Melcher, K., & He, S. Y. (2015). Structural basis of JAZ repression of MYC transcription factors in jasmonate signalling. *Nature* *2015* *525*:7568, *525*(7568), 269–273. <https://doi.org/10.1038/nature14661>
- Zhang, X., Yan, F., Tang, Y., Yuan, Y., Deng, W., & Li, Z. (2015). Auxin Response Gene SIARF3 Plays Multiple Roles in Tomato Development and is Involved in the Formation of Epidermal Cells and Trichomes. *Plant and Cell Physiology*, *56*(11), 2110–2124. <https://doi.org/10.1093/PCP/PCV136>
- Zhang, Y., Shen, J., Bartholomew, E. S., Dong, M., Chen, S., Yin, S., Zhai, X., Feng, Z., Ren, H., & Liu, X. (2021). TINY BRANCHED HAIR functions in multicellular trichome development through an ethylene pathway in *Cucumis sativus* L. *The Plant Journal*, *106*(3), 753–765.
- Zhang, Z., Chen, X., Guan, X., Liu, Y., Chen, H., Wang, T., Mouekouba, L. D. O., Li, J., & Wang, A. (2014). A genome-wide survey of homeodomain-leucine zipper genes and analysis of cold-responsive HD-Zip I members' expression in tomato. *Bioscience, Biotechnology, and Biochemistry*, *78*(8), 1337–1349. <https://doi.org/10.1080/09168451.2014.923292>
- Zhao, J.-L., Pan, J.-S., Guan, Y., Zhang, W.-W., Bie, B.-B., Wang, Y.-L., He, H.-L., Lian, H.-L., & Cai, R. (2015). Micro-trichome as a class I homeodomain-leucine zipper gene regulates multicellular trichome development in *Cucumis sativus*. *Journal of Integrative Plant Biology*, *57*(11), 925–935. <https://doi.org/https://doi.org/10.1111/jipb.12345>
- Zhao, P., Li, Q., Li, J., Wang, L., & Ren, Z. (2014). Genome-wide identification and characterization of R2R3MYB family in *Solanum lycopersicum*. *Molecular Genetics and Genomics*, 1183–1207. <https://doi.org/10.1007/s00438-014-0879-4>
- Zheng, F., Cui, L., Li, C., Xie, Q., Ai, G., Wang, J., Yu, H., Wang, T., Zhang, J., Ye, Z., & Yang, C. (2022). Hair interacts with SIZFP8-like to regulate the initiation and elongation of trichomes by modulating SIZFP6 expression in tomato. *Journal of Experimental Botany*, *73*(1), 228–244. <https://doi.org/10.1093/jxb/erab417>
- Zhou, F., & Pichersky, E. (2020). The complete functional characterisation of the terpene synthase family in tomato. *New Phytologist*, *226*(5), 1341–1360. <https://doi.org/10.1111/nph.16431>
- Zhou, S., Hu, Z., Li, F., Yu, X., Naeem, M., Zhang, Y., & Chen, G. (2018). Manipulation of plant architecture and flowering time by down-regulation of the GRAS transcription factor SIGRAS26 in *Solanum lycopersicum*. *Plant Science*, *271*, 81–93.

4.9 Supplemental data

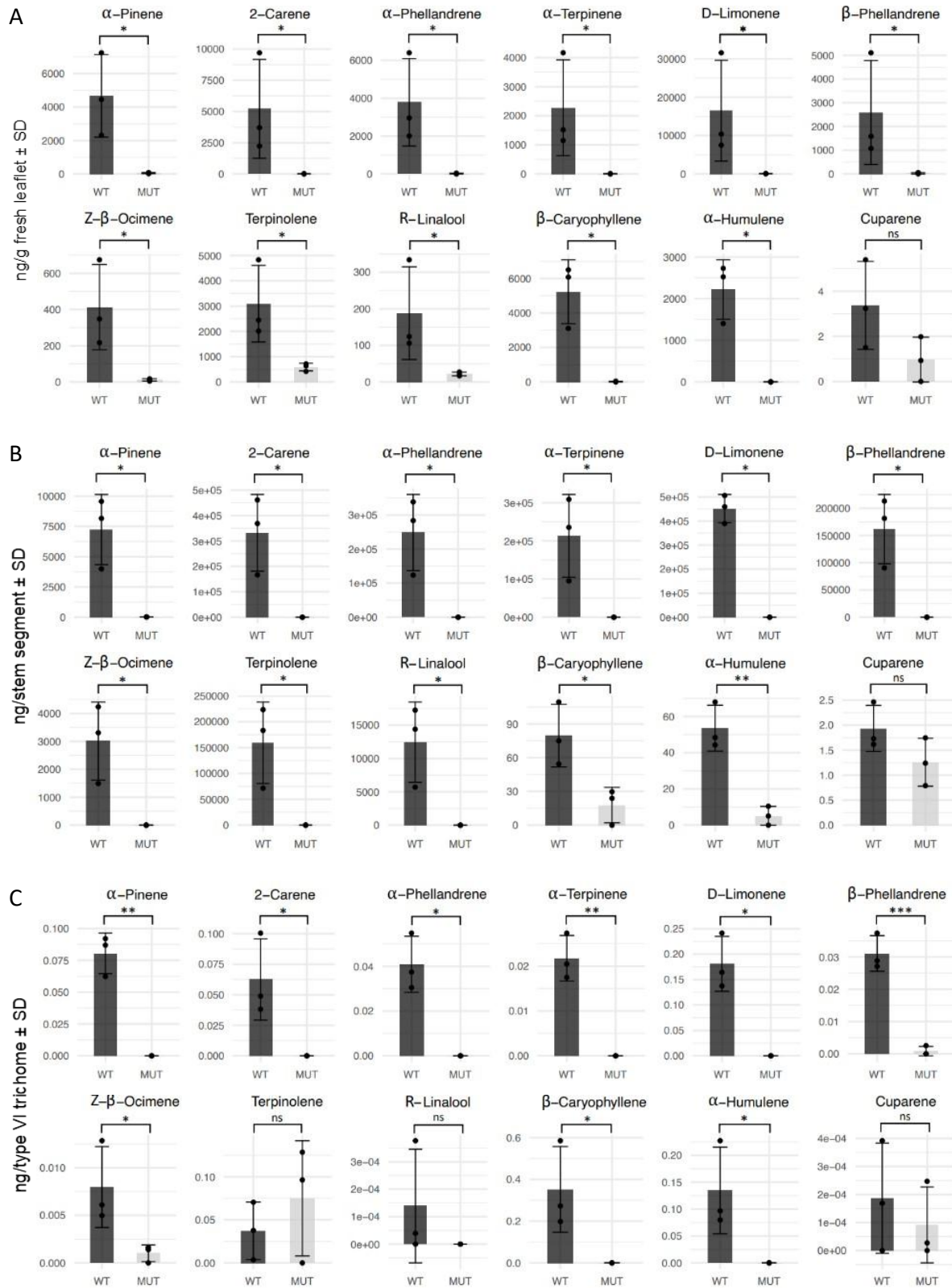




Supplemental Figure 1 Maps of the plasmids used to generate CRISPR mutants. Three sgRNAs were multiplexed in sequence, each one under the control of the *Arabidopsis thaliana* class III RNA polymerase U6 promoter, with the addition of attL5 and attL2 gateway sites respectively at the 5' and 3' end of the construct and cloned into pUC57 vector generating pUC57-CC-HDZ38 (A). This was recombined, together with Cas9 entry vector pYPQ150 (B) with the destination vector pK2GW7 (C) in a multi-site reaction via Gateway® LR clonase® II producing pK2GW7-CC-HDZ38 (D).



Supplemental Figure 2 Leaf trichomes densities. Type I and III (A), type V (B), type VI (C) and type VII (D) trichomes densities on adaxial (AD) and abaxial (AB) sides of glandless mutant (MUT) and wild-type (WT) leaves. The bars represent the mean values \pm standard deviation (SD) of each trichome type density, calculated from scanning-electron micrographs (0.6 cm diameter) of leaves from different plants ($n=3$). Differences in bars are annotated accordingly to the results of independent *t*-tests after Shapiro-Wilk's normality test and *F*-test comparison of variances (* $p < 0.05$, ** $p < 0.01$, ns non-significant). Significance for non-normally distributed subset was assessed with Wilcoxon non-parametric test.

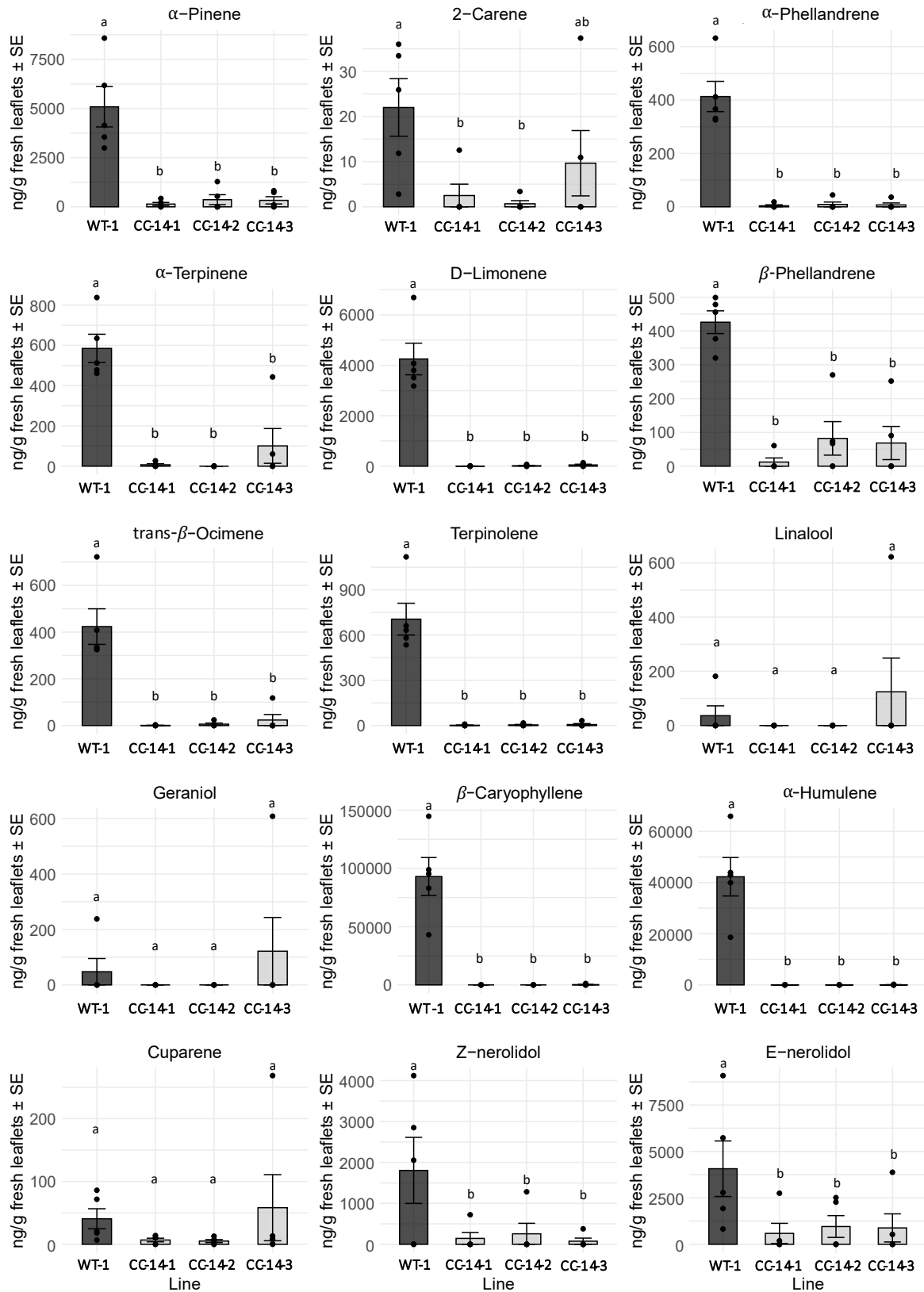


Supplemental Figure 3 Volatile mono- and sesquiterpenes in leaves, stems and isolated type VI trichomes. Mono- and Sesquiterpene levels in leaves (A), stems (B) and isolated type VI trichomes (C) of wild-type (WT) and glandless mutant (MUT) tomato plants (n=3). The bars represent the mean values \pm standard deviation (SD) of target volatile terpene level quantified by GC-MS and normalized by stem length, leaf fresh weight and number of isolated type VI trichomes. Differences in bars are annotated according to the significance levels resulting from independent t-tests after Shapiro-Wilk's normality test and F-test comparison of variances (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ns non-significant). Significance for non-normally distributed subset was assessed with Wilcoxon non-parametric test.

Chapter 4 – SIHDZ38 regulates type VI trichome development and trichome densities



Supplemental Figure 5 Effect of CRISPR-Cas9 mutations on SIHDZ38 protein. Amino acid sequence alignment of SIHDZ38 as encoded by the WT Solyc09g008810 gene, and by the edited-version of the gene in three selected independent the CRISPR-Cas9 mutant lines. Non conserved amino acids are marked (*). Predicted functional domains are annotated under the sequence alignment.



Supplemental Figure 6 Volatile mono- and sesquiterpenes in leaves. Mono- and Sesquiterpene levels in leaves of wild-type (WT-1) and three independent T2 CRISPR-Cas9 SIHDZ38 gene-edited lines (CC-14-1, CC-14-2, CC-14-3). The bars represent the mean values \pm standard error (SE) of target volatile terpene level quantified by GC-MS and normalized by leaf fresh weight. Significant differences in bars are annotated with different letters according to Fisher's LSD test after one-way ANOVA ($p < 0.05$).

Chapter 4 – SIHDZ38 regulates type VI trichome development and trichome densities

Primers for constructs generation, sequencing and RT-qPCR		
Gene/Construct name	Primer name	Primer sequence
SIHDZ38 (Solyc09g008810)	F_CDS_ZHD38	ATGGATTGGAATGGAAATATTAGACC
	R_CDS_ZHD38	TCATGGATATGTTGGCAATGTG
	F_qPCR_ZHD38_1	ACAACCAAGACAAATTGCTGTTTGG
	R_qPCR_ZHD38_1	TGCCAATACCTCATCTTGAAGCTT
	F_qPCR_ZHD38_2	AGATGAGGTATTGGCATTAAAGAGCA
	R_qPCR_ZHD38_2	TGCTTTGACTGTTTCTTCACCT
	F_qPCR_ZHD38_3	AGGTGAAGAAACAGTCGAAAGCAC
	R_qPCR_ZHD38_3	CATGGATATGTTGGCAATGTGGC
	seq_ZHD38_1	CTCCTTTGCCACTAAATTAGGA
	seq_ZHD38_2	TGGATTATTGAGTTTTTCTCGACAT
	seq_ZHD38_3	TGTCGAGAAAAACTCAATAATCC
	seq_ZHD38_4	GGTCTTGAGTTTTCTGTTATGGA
	seq_ZHD38_5	TTCTTTTACCACCTTCACTTGATGA
	seq_ZHD38_6	GGGACCTTGAGTGGGTGACT
	seq_ZHD38_7	AAATCCCCAATTAATTAACAGCTT
	seq_ZHD38_8	TCACGTAAAATGAGACAAAAGTGC
seq_ZHD38_9	GATTTTCAGCCACGCAAATA	
seq_ZHD38_10	AGGCAAAATTTCAAGAAAACACA	
seq_ZHD38_11	TCAAACACAAAATAGCCGATTA	
pK2GW7-CC-HDZ38	R_Gat_RB_2979	GCACATACAAATGGACGAACG
	R_t35S_3252	GATTTGTAGAGAGAGACTGGTG
	F_Cas9	ACGAGCTTGCTCTTCCATCT
	F_p35S_4218	AGAGGACTCCGGTATTTTTACAAC
	R_p35S_4983	CTTGCGAAGGATAGTGGGATTG
R_Cas9_9655	GCTTGTGCTGCTCAACGAAA	
pTRV-2b-HDZ38	R_TRV2b_4857	CTTCAGACACGGATCTAC
	F_TRV2b_4858	GGAGAATCGGGACTTCAGACT
sgRNA sequences		
Gene name	sgRNA sequence	
<i>SIHDZ38 (Solyc09g008810)</i>	GCAATGCAAACACAACATGG	
	AGACTATGATGTTGTCTCAA	
	TCCAAAATAGAAGAGCTAGA	
VIGS target		
Gene name	Target sequence	
<i>SIHDZ38 (Solyc09g008810)</i>	GGATTGGAATGGAAATATTAGACCTTTTGTTCGTTTCAAGACAAGTTATTGATAATTCTCTCAATTTTCTTACAAC ACAATTATGACCAATATCCAGGTATTGAAATGAAGCATGCAATGCAAACACAACATGGTGGAGTACAAGTCC CAACAATGGACAACAACAACAACAACCTTTGTACTCAATCAACATCAATTGGACAAGAAGAAAAGTTGTCAA GTGATCAATTAGAGTCACCTTGAGAATAGTTTTCAAGAAGAGATAAACTTGATCCAGACAGGAAAATGAAAT TGGCTAAAGA	
Oligo sequences for Gateway® cloning		
Sequence name	Sequence	
attB1	GGGACAAGTTTGTACAAAAAGCAGGCTNN	
attB2	GGGACCACCTTTGTACAAGAAAGCTGGGTN	
attL1	CAAATAATGATTTTATTTGACTGATAGTGACCTGTTCTGTTGCAACAAATTGATGAGCAATGCTTTTTATAAT	
attL2	ACCCAGCTTTCTGTACAAAGTTGGCATTATAAGAAAGCATTGCTTATCAATTTGTTGCAACGAACAGGTCAC	
attL5	CCCAAATAATGATTTTATTTGACTGATAGTGACCTGTTCTGTTGCAACAAATTGATGAGCAATGCTTTTTATA	

Supplemental table 1 Primers used in this study