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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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Summary

Plant trichomes are specialized epidermal structures that mediate various ecological interactions through their morphological and metabolic versatility. In particular, glandular trichomes possess the unique ability to biosynthesize and secrete specialized metabolites such as terpenoids, phenylpropanoids, and acylsugars, which contribute to defense against herbivores, attraction of their enemies, or protection from abiotic stress. Among cultivated crops, *Solanum lycopersicum* (tomato) is a model crop for trichome research, due to the diversity of its trichome types and their specialized metabolism.

Type VI glandular trichomes in tomato are characterized by a head of four gland cells that are the primary site for volatile terpene biosynthesis. These terpenes, including mono-, sesqui-, and diterpenes, are produced by a suite of terpene synthases (TPSs), whose expression is regulated by transcription factors such as *SIMYC1* (a bHLH transcription factor) and *SIWO* (a HD-ZIP IV transcription factor). The expression and activity of these regulators are modulated by jasmonic acid (JA) and JAZ (JASMONATE ZIM DOMAIN) transcriptional repressor proteins. The precise regulatory network upstream of *SIMYC1* and downstream targets involved in controlling trichome density, identity, and metabolism are still not fully known. This thesis addresses these gaps by exploring the transcriptional network that governs both the developmental and metabolic traits of type VI trichomes, with a particular focus on *SIMYC1* as a master regulator.

Chapter 2 presents the identification and characterization of *SIZHD18*, a Zinc-finger Homeodomain (ZHD) transcription factor, as a putative upstream regulator of *SIMYC1*. Using yeast-one-hybrid screening, *SIZHD18* was shown to bind the *SIMYC1* promoter. Functional validation via virus-induced gene silencing (VIGS) and CRISPR-Cas9 knockout lines revealed that knock-down or -out of *SIZHD18* significantly reduced the density of type VI trichomes on both leaves and stems and alters the profile of volatile sesquiterpenes. This suggests a direct role for *SIZHD18* in regulating both development and metabolism. Interestingly, *SIMYC1* was also found to bind its own promoter, indicating a possible autoregulatory feedback loop. Together, these results propose a regulatory cascade wherein *SIZHD18* activates *SIMYC1*, which in turn may sustain or amplify its own expression, leading to robust induction of trichome-specific terpene biosynthesis genes.

To further decipher the regulatory network downstream of *SIMYC1*, in Chapter 3 we studied transcriptional changes in transgenic tomato lines overexpressing *SIMYC1*, or in which *SIMYC1* was knocked down, or knocked out. Differential gene expression analyses highlighted several putative target TFs, three of which—*SlbHLH114*, *SIMYB14*, and *SIWRKY1*—were selected for detailed functional studies. Phenotypic characterization of CRISPR-Cas9 knockout lines showed that *SlbHLH114* and *SIWRKY1* act as positive regulators of type VI trichome density but concurrently suppress the expression of certain terpene synthases. This suggests a division of labor within the *SIMYC1*-regulated module, where some TFs prioritize trichome development while inversely modulating metabolite production. Instead, *SIMYB14* appeared to play a more minor or redundant role in these processes. Additionally, *SIMYC1* expression was found to influence also primary metabolism and basal defense responses, reinforcing its position as a central integrator of developmental and physiological signaling within trichomes.

In Chapter 4, we investigated the *glandless* tomato mutant, which lacks the four characteristic glandular cells from type VI trichomes. Genetic mapping and reverse genetics approaches identified the causal mutation in *SIHDZ38*, a class I HD-ZIP transcription factor. This was confirmed by transiently knocking down *SIHDZ38* via VIGS. CRISPR-Cas9 knockout of *SIHDZ38* did not phenocopy exactly the *glandless* trait, but determined the complete loss of type VI glandular trichomes, confirming its essential role in the development of this type of trichomes.

Transcriptomic analysis of SIHDZ38 knockout lines revealed broad downregulation of trichome-specific TFs and terpene synthase genes, placing SIHDZ38 as a key determinant of glandular identity and terpene metabolism in type VI trichomes. The discovery of this transcription factor adds an entirely new layer to the regulatory hierarchy, with SIHDZ38 acting either in parallel with or upstream of SIWO and SIMYC1.

In the final discussion in Chapter 5 all our findings are integrated into updated and coherent regulatory models of type VI trichome development and specialized metabolism. SIZHD18 is proposed as a positive upstream regulator of SIMYC1, which serves as a central node coordinating both developmental and metabolic gene networks. Downstream effectors such as SlbHLH114 and SIWRKY1 further refine type VI trichome density and specialized metabolism. Independently, SIHDZ38 governs type VI trichome development and glandular cells differentiation and contribute to the regulation of the specialized metabolism via regulation of other trichome-associated TFs.

Importantly, this thesis illustrates the intricate interplay between transcription factor cascades, transcriptional feedback loops, and jasmonic acid signaling. The identification of new key regulators like SIZHD18 and SIHDZ38 not only enhances our fundamental understanding of trichome biology but also paves the way for metabolic engineering aimed at improving pest and stress resistance and boosting the production of valuable phytochemicals in tomato and other Solanaceous crops.

Samenvatting

Plantaardige trichomen zijn gespecialiseerde epidermale structuren die dankzij hun morfologische en metabolische veelzijdigheid een breed scala aan ecologische interacties mogelijk maken. In het bijzonder beschikken glandulaire trichomen – klierharen - over het unieke vermogen om gespecialiseerde metabolieten te synthetiseren en te secreteren, zoals terpenoïden, fenyylpropanoïden en acylsuikers. Deze verbindingen dragen bij aan de verdediging tegen herbivoren, de aantrekking van hun vijanden en bescherming tegen abiotische stress. Onder de gecultiveerde gewassen is *Solanum lycopersicum* (tomaat) een modelgewas voor trichoomonderzoek, vanwege de diversiteit aan trichoomtypen en hun gespecialiseerde metabolisme.

Type VI klierharen in tomaten worden gekenmerkt door een apicale klier bestaande uit vier cellen, die fungeren als de primaire locatie voor de biosynthese van vluchtige terpenen. Deze terpenen, waaronder mono-, sesqui- en diterpenen, worden geproduceerd door een reeks terpeensynthasen (TPSs), waarvan de expressie gereguleerd wordt door transcriptiefactoren zoals SIMYC1 (een bHLH-transcriptiefactor) en SIWO (een HD-ZIP IV-transcriptiefactor). De expressie en activiteit van deze regulatoren worden op hun beurt gemoduleerd door jasmonzuur (JA) en JAZ-transcriptie repressoren. Het precieze regulatienetwerk stroomopwaarts (upstream) van SIMYC1 en de stroomafwaarts (downstream) doelen die betrokken zijn bij de controle van trichoomdichtheid, identiteit en metabolisme zijn nog niet volledig opgehelderd. Dit proefschrift tracht deze lacunes te adresseren door het transcriptienetwerk te onderzoeken dat zowel de ontwikkelings- als metabolische eigenschappen van type VI-trichomen aanstuurt, met bijzondere aandacht voor SIMYC1 als hoofdregulator.

Hoofdstuk 2 beschrijft de identificatie en karakterisering van SIZHD18, een Zinc-finger Homeodomein (ZHD) transcriptiefactor, als een vermoedelijke upstream regulator van SIMYC1. Middels een yeast-one-hybrid screen werd aangetoond dat SIZHD18 bindt aan de promotor van SIMYC1. Functionele validatie via virus-geïnduceerde onderdrukking van genexpressie (VIGS) en CRISPR-Cas9 knock-out lijnen toonde aan dat het verlagen of uitschakelen van SIZHD18 leidt tot een significante vermindering van de dichtheid van type VI-trichomen op zowel bladeren als stengels, en tot veranderingen in het profiel van vluchtige sesquiterpenen. Dit suggereert een directe rol voor SIZHD18 in de regulatie van zowel trichoom ontwikkeling als metabolisme.

Opmerkelijk is dat ook SIMYC1 zijn eigen promotor bleek te binden, wat wijst op een mogelijke autoregulerende terugkoppeling. Gezamenlijk suggereren deze resultaten een regulerende cascade waarin SIZHD18 SIMYC1 activeert, die vervolgens mogelijk zijn eigen expressie handhaaft of versterkt, wat leidt tot een krachtige inductie van trichoomspecifieke terpeenbiosynthesegenen.

Om het regulatienetwerk downstream van SIMYC1 verder te ontrafelen, onderzochten we in Hoofdstuk 3 transcriptieveranderingen in transgene tomatenplanten die SLMYC1 tot overexpressie brengen of waarvan de expressie van SIMYC1 onderdrukt of uitgeschakeld is. Differentiële genexpressieanalyses leidden tot de selectie van verschillende doelwit-transcriptiefactoren, waarvan er drie—SibHLH114, SIMYB14 en SIWRKY1—werden gekozen voor diepgaand functioneel onderzoek.

Fenotypische karakterisering van CRISPR-Cas9 knock-out lijnen toonde aan dat SibHLH114 en SIWRKY1 optreden als positieve regulatoren van de dichtheid van type VI-trichomen, maar tegelijkertijd de expressie van bepaalde terpeen synthase genen onderdrukken. Dit wijst op een taakverdeling binnen het door SIMYC1 gereguleerde netwerk, waarbij sommige transcriptiefactoren prioriteit geven aan trichoomontwikkeling, terwijl zij omgekeerd de productie van metabolieten beïnvloeden. SIMYB14 daarentegen bleek een ondergeschikte of redundante rol te spelen. Bovendien

bleek SIMYC1 ook invloed uit te oefenen op het primaire metabolisme en de basale afweer, wat haar centrale rol als integrator van ontwikkelings- en fysiologische signalering in trichomen onderstreept.

In Hoofdstuk 4 onderzochten we de *glandless* tomatenmutant, die de vier karakteristieke kliercellen van type VI-trichomen mist. Genetische mapping identificeerde een mutatie in SIHDZ38, een HD-ZIP transcriptiefactor van klasse I, als oorzaak, gevolgd door een bevestiging van het fenotype met VIGS. CRISPR-Cas9 knock-out van SIHDZ38 resulteerde niet in een exact fenokopie van de *glandless*-mutant, maar leidde tot een volledig verlies van type VI glandulaire trichomen, waarmee de essentiële rol van SIHDZ38 in de ontwikkeling van dit type trichomen werd bevestigd.

Transcriptomische analyse van SIHDZ38 knock-out lijnen toonde een brede neerwaartse regulatie van transcriptiefactoren belangrijk voor trichoom ontwikkeling en TPS-genen, wat SIHDZ38 positioneert als een sleutelregulator van klieridentiteit en terpeenmetabolisme in type VI-trichomen. De ontdekking van deze factor voegt een nieuwe dimensie toe aan de regulatoire hiërarchie, waarbij SIHDZ38 mogelijk functioneert in parallel met of upstream van SIWO en SIMYC1.

In de afsluitende discussie van Hoofdstuk 5 worden alle bevindingen geïntegreerd in geactualiseerde en coherente regulatiemodellen voor de ontwikkeling en het gespecialiseerde metabolisme van type VI-trichomen. SIZHD18 wordt voorgesteld als een positieve upstream regulator van SIMYC1, die fungeert als een centraal knooppunt dat zowel ontwikkelings- als metabolische genennetwerken coördineert. Downstream transcriptiefactoren zoals SlbHLH114 en SIWRKY1 verfijnen de dichtheid en het metabolisme van type VI-trichomen verder. Onafhankelijk daarvan reguleert SIHDZ38 de ontwikkeling van type VI-trichomen en de differentiatie van kliercellen, en draagt bij aan de regulatie van het gespecialiseerde metabolisme via andere trichoom-gerelateerde transcriptiefactoren.

Belangrijk is dat dit proefschrift de complexe wisselwerking aantoont tussen transcriptiefactor-cascades, transcriptie-feedbackmechanismen en jasmonzuursignalering. De identificatie van nieuwe sleutelregulatoren zoals SIZHD18 en SIHDZ38 verrijkt niet alleen het fundamenteel begrip van trichoombiologie, maar opent ook de weg naar metabolische engineering ter verbetering van plaag- en stressresistentie, en voor de verhoging van de productie van waardevolle fytochemicaliën in tomaat en andere Solanaceae.

List of Publications

Zocca, P., van Doore, E., Roovers, A.J.M., Glas, J.J., Uittenbogaard, M., Verlaan, M.G., van Herwijnen, Z., Haring, M.A. and Schuurink, R.C. (2025), *Glandless*, a tomato HD-ZIP transcription factor, is important for the gland formation of type VI trichomes. *Plant J*, 123: e70308. <https://doi.org/10.1111/tpj.70308>.

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