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Solanoeclepin A

Characterization of a rhizosphere communication molecule in tomato and potato

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Summary

In **Chapter 1** of my thesis, I introduce the concept of the soil ecosystem and its importance for agriculture. I explain how plants shape their rhizosphere using signaling molecules secreted by their roots, focusing the attention on different types of interactions between plants and the surrounding organisms including microbes, fungi, nematodes and other plants. Both beneficial and harmful types of interactions are explained, including symbiotic relationships, allelopathy and tritrophic interactions. I give special attention to the plant-nematode interaction, introducing the life cycle of plant parasitic nematodes and introducing the concept of eclepins. Eclepins are triterpenoid molecules secreted into the rhizosphere, which induce hatching in parasitic cyst nematodes such as potato cyst nematodes (PCNs). The most active PCN hatching factor is Solanoeclipin A (SoLA) found in potato and tomato root exudate. The presence of such molecules in the rhizosphere opens the possibility of exploitation of these signaling cues to improve agriculture, but the function of the majority of molecules in the root exudate is still unknown.

The role of SoLA as PCN hatching factor has been studied since 1949 when its molecular structure was not identified yet and it was referred to as ‘potato cyst nematode hatching factor’. Since the discovery of the SoLA structure in 1996, studies of this molecule have been mostly focused on its chemical synthesis and the interaction with PCNs. Since the beneficial function of SoLA in the rhizosphere has not been clarified yet, and its study is hindered by the low concentration in the root exudates, in **Chapter 2** I describe an efficient analytical method to detect SoLA in root exudates with a single-step extraction and UPLC-MS/MS analysis. I used the method to detect SoLA in different wild tomato relatives and accessions including the trap crop *Solanum sisymbriifolium* and demonstrated there is quite some variation in the level of SoLA produced by different genotypes. Using hatching assays with PCN *Globodera pallida*, I demonstrate a positive correlation between the SoLA concentration in the root exudate and the hatching rate.

The detection of SoLA in wild tomato relatives in **Chapter 2**, prompted me to screen a collection of 340 wild potato relatives belonging to the *Petota* section for their SoLA production in **Chapter 3**. I found a range of species producing SoLA, but also a large number that did not. Even within the same species there was variation between accessions that produce SoLA and others that do not. Hatching assays with *G. pallida* showed that some of the accessions that do not produce SoLA still induce PCN hatching hinting at the presence of other hatching factors (HFs) or hatching stimulants (HSs) like SoLA. To investigate the nature of these new signaling molecules, fractionation of a selection of root exudates was performed and hatching of the different fractions was assessed. Also, this suggests that other HFs are present in the root exudates with a potential hatching activity higher than SoLA. Interestingly, *Globodera rostochiensis* showed quite a different response to these wild potato root exudates than *G. pallida*, suggesting that the two *Globodera* species have different sensitivity to the same HFs or inhibitors.

To be able to characterize SoLA biosynthetic gene candidates, in **Chapter 4** I developed systemic Virus Induced Gene Silencing (VIGS) to silence genes involved in terpenoid pathways expressed in the roots. As model candidate genes, I used *STEROL*

METHYLTRANSFERASE (SMT1) and *STEROL SIDE CHAIN REDUCTASE 2 (SSR2)* involved in the biosynthesis of phytosterols and steroidal glycoalkaloids, respectively. Silencing of both genes, in the roots, was successful, and upon *SMT1* silencing the phytosterol concentration in both root and root exudate were reduced. Silencing of *SSR2* not only reduced biosynthesis of steroidal glycoalkaloids, but also of saponins, while, surprisingly, the concentration of SolA increased. Hatching assays with *G. rostochiensis* confirmed this observation. Since the silenced plants displayed quite different root exudate metabolic profiles, I carried out microbiome analysis and demonstrated changes in the microbial community composition.

In **Chapter 5** I studied the biosynthesis of SolA using a custom-build aeroponics system. I found that the SolA concentration in tomato root exudate is influenced by abiotic and biotic factors, in particular, nitrogen deficiency and the presence of microbes increased it. Following these results, I performed an RNA-seq experiment on the roots of the tomato plants in order to identify genes that correlate with SolA production. I found four possible candidate genes including a terpene cyclase and three cytochrome P450s.

Finally, in **Chapter 6** I discuss the findings of my thesis, including the importance of a reliable analytical method to quantify the SolA concentration in root exudates and how biotic and abiotic factors stimulate its production. Moreover, I discuss the use of VIGS as a great tool to investigate and validate candidate genes involved in the biosynthesis of SolA and other root produced metabolites and the role these metabolites may have on rhizosphere organisms.

Samenvatting

In **Hoofdstuk 1** van mijn thesis introduceer ik het concept van het bodemecosysteem en het belang ervan voor de landbouw. Ik leg uit hoe planten hun rhizosfeer vormen met behulp van signaalmoleculen die door hun wortels worden uitgescheiden, waarbij ik de aandacht vestig op verschillende soorten interacties tussen planten en de omringende organismen, waaronder microben, schimmels, nematoden en andere planten. Zowel gunstige als schadelijke soorten interacties worden uitgelegd, waaronder symbiotische relaties, allelopathie en tritrofische interacties. Ik besteed speciale aandacht aan de plant-nematoden interactie, het introduceren van de levenscyclus van plantparasitaire nematoden en het introduceren van het concept van eclepinen. Eclepinen zijn triterpenoïde moleculen die door planten in de rhizosfeer worden uitgescheiden en die het uit het ei komen van parasitaire cystenaaltjes zoals aardappelcystenaaltjes (PCN's) induceren. Het eclepine van PCN heet solanoeclepin A (solA), dat wordt aangetroffen in exudaat van aardappel- en tomatenwortels. De aanwezigheid van dergelijke moleculen in de rhizosfeer opent de mogelijkheid om deze stoffen te gebruiken om de landbouw te verbeteren, maar de functie van de meeste moleculen in het wortelxudaat is nog onbekend.

De rol van SolA is onderzocht sinds 1949, toen de moleculaire structuur ervan nog niet was geïdentificeerd en het werd aangeduid als aardappelcystenematode 'hatching' factor. Sinds de ontdekking van de SolA-structuur in 1996 zijn studies van dit molecuul vooral gericht op de chemische synthese en de interactie met PCNs. Aangezien een eventuele gunstige functie van SolA in de rhizosfeer nog niet is opgehelderd, en de studie daarvan wordt belemmerd door de lage concentratie in de wortelxudaten, beschrijf ik in **Hoofdstuk 2** een efficiënte analytische methode om SolA in wortelxudaten te detecteren met een extractie in één stap. en UPLC-MS/MS-analyse. Ik heb de methode gebruikt om SolA te analyseren in een aantal wilde tomaten verwanten, waaronder de 'trapcrop' *Solanum sisymbriifolium*, en ik heb aangetoond dat er nogal wat variatie is in het niveau van SolA dat wordt geproduceerd. Met behulp van hatching-assays met PCN *Globodera pallida*, demonstreer ik een positieve correlatie tussen de SolA-concentratie in het wortelxudaat en de hoeveelheid larven die uit het ei komen.

De detectie van SolA bij verwante wilde tomaten in **Hoofdstuk 2** was voor mij aanleiding om in **Hoofdstuk 3** een verzameling van 340 verwante wilde aardappelverwanten uit de *Petota*-sectie te screenen op hun SolA-productie. Ik vond een reeks soorten die SolA produceerden, maar ook een groot aantal die dat niet deden. Zelfs binnen dezelfde soort was er variatie tussen genotypes die SolA produceren en andere die dat niet doen. Tests met *G. pallida* toonden aan dat sommige van de genotypes die geen SolA produceren nog steeds PCN 'hatching' induceren, wat waarschijnlijk duidt op de aanwezigheid van andere 'hatching' factoren (HFs) of 'hatching stimulants' (HSs). Om de aard van deze nieuwe signaalmoleculen te onderzoeken, werd fractionering van een selectie van wortelxudaten uitgevoerd en werd de 'hatching' activiteit van de verschillende fracties beoordeeld. Ook dit suggereert dat andere HFs aanwezig zijn in de wortelxudaten. Interessant is dat *Globodera rostochiensis* een heel andere reactie op deze wilde aardappelwortelxudaten

vertoonde dan *G. pallida*, wat suggereert dat de twee *Globodera*-soorten een verschillende gevoeligheid hebben voor dezelfde HF's.

Om in staat te zijn SolA biosynthetische genkandidaten te karakteriseren, heb ik in **Hoofdstuk 4** systemische Virus Induced Gene Silencing (VIGS) ontwikkeld om de expressie van genen die betrokken zijn bij terpenoïde routes in de wortels, te verlagen. Als model kandidaatgenen gebruikte ik *STEROL METHYLTRANSFERASE (SMT1)* en *STEROL SIDE CHAIN REDUCTASE 2 (SSR2)* die betrokken zijn bij de biosynthese van respectievelijk fytosterolen en steroïdale glycoalkaloïden. Silencing van deze genen, in de wortels, was succesvol, en na *SMT1* silencing was de fytosterolconcentratie in zowel wortel- als wortellexudaat verminderd. Silencing van *SSR2* verminderde niet alleen de biosynthese van steroïde glycoalkaloïden, maar ook van saponinen, terwijl verrassend genoeg de concentratie van SolA toenam. 'Hatching' tests met *G. rostochiensis* bevestigden deze waarneming. Omdat de op deze manier behandelde planten heel verschillende wortellexudaat metabolische profielen vertoonden, heb ik microbiom analyses uitgevoerd en veranderingen in de samenstelling van de microbiële gemeenschap aangetoond.

In **Hoofdstuk 5** heb ik de biosynthese van SolA bestudeerd met behulp van een zelf ontwikkeld aeroponics systeem. Ik ontdekte dat de SolA-concentratie in het exudaat van tomatenwortels wordt beïnvloed door abiotische en biotische factoren, met name stikstofgebrek en de aanwezigheid van microben. Naar aanleiding van deze resultaten heb ik een RNA-seq-experiment uitgevoerd op de wortels van de tomatenplanten om genen te identificeren die correleren met SolA-productie. Ik vond vier mogelijke kandidaatgenen, waaronder een terpeencyclase en drie cytochroom P450's.

Ten slotte bespreek ik in Hoofdstuk 6 de bevindingen van mijn proefschrift, waaronder het belang van een betrouwbare analytische methode om de SolA-concentratie in wortellexudaten te kwantificeren en hoe biotische en abiotische factoren de productie ervan stimuleren. Bovendien bespreek ik het gebruik van VIGS als een geweldig hulpmiddel voor het onderzoeken en valideren van kandidaatgenen die betrokken zijn bij de biosynthese van SolA en andere door wortels geproduceerde metabolieten en de rol die deze metabolieten kunnen hebben op rhizosfeer-organismen.

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List of Publications

Guerrieri, A., Dong, L., & Bouwmeester, H. J. (2019). Role and exploitation of underground chemical signaling in plants. *Pest management science*, 75(9), 2455-2463.

Guerrieri, A., Floková, K., Vlaar, L. E., Schilder, M. L., Kramer, G., Chojnacka, A., van Dijk, Y. R., Bouwmeester, H. J., & Dong, L. (2021). UPLC-MS/MS analysis and biological activity of the potato cyst nematode hatching stimulant, solanoecelepin A, in the root exudate of *Solanum* spp. *Planta*, 254(6), 1-13.

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Guerrieri, A., Abedini, D., White, F., Kramer, G., Bleeker, J., Bouwmeester, H. J., & Dong, L. Virus-induced gene silencing of key genes in the biosynthesis of cycloartenol-derived compounds changes the root metabolome and microbiome composition in tomato. (In preparation)

Guerrieri, A., Abedini, D., White, F., Yang, Y., Bouwmeester, H. J., & Dong, L. Nitrogen deficiency and microorganisms induce solanoecelepin A production in tomato. (In preparation)

Moglia, A., Florio, F. E., Iacopino, S., **Guerrieri, A.,** Milani, A. M., Comino, C., et al. (2020). Identification of a new R3 MYB type repressor and functional characterization of the members of the MBW transcriptional complex involved in anthocyanin biosynthesis in eggplant (*S. melongena* L.). *PLoS One*, 15(5), e0232986.