Elucidation of the biosynthetic pathway and biological roles of strigolactones in maize and rice

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Summary
Strigolactones (SLs) are a class of plant signaling molecules of great importance, with diversified structures and diverse biological roles in and outside the plant, in the rhizosphere. In Chapter 1, I introduce the SLs, and review their discovery and biological functions, the regulation of their production by nutrient availability, and their perception and downstream signaling. I particularly emphasize the biosynthesis of SLs, including carlactone biosynthesis and the structural diversification of the SLs generated in the biosynthetic pathways downstream of carlactone. In this process of SL structural diversification, a range of enzymes such as cytochrome P450s are involved of which many are still unknown. I pay attention to possible approaches of SL biosynthetic gene discovery and characterization of their function.

Parasitic plants employ a haustorium to connect to the vasculature of their host plants, through which they then absorb water, assimilates, and nutrients. As root parasitic plants are obligate parasites, depending completely on a host for their survival, they need to closely coordinate their lifecycle with that of their host. Here, parasitic plants have evolved a number of host detection/host response mechanisms. In Chapter 2 the germination stimulants, triggering germination of the Orobanchaceae, one major parasitic plant family, are reviewed, in which SLs are the major class. We review how these compounds are produced and in which host plants. And we discuss why they are reliable signals, how parasitic plants have evolved mechanisms that detect and respond to them, and whether they play a role in host specificity. The knowledge underlying this signaling relationship between host and parasitic plant will improve our understanding of the evolution of plant parasitism and will facilitate the development of more effective control measures in cases where these parasitic plants have developed into weeds.

In Chapter 3 and 4, I elucidated the biosynthetic pathway and biological functions of maize and rice SLs. Maize (Zea mays) is one of the most important staple crops in the world. However, in Africa, its yield is severely compromised by the parasitic witchweeds, Striga hermonthica and Striga asiatica. Maize roots exude at least six SLs but only two of them were structurally identified when I started my PhD project. The identity of the other maize SLs, as well as their role in Striga germination and their biosynthetic origin, all remained elusive. In Chapter 3, by using a combination of approaches, including co-expression analysis and (transient) gene expression in Nicotiana benthamiana and yeast, I revealed natural variation in the maize SL production, identified three new maize SLs, and elucidated the biosynthetic pathway of the maize SLs. We discovered a biosynthetic gene cluster for zealactone biosynthesis and a novel cytochrome P450, ZmCYP706C37, catalyzing several steps in the biosynthesis of maize SLs. Among these SLs, zealactol and zealactonoic acid showed much lower activity than zealactone, in inducing Striga germination. I also showed that changes in the composition of the SL blend in some mutant lines correspond to differences in Striga germination, and, as a consequence, Striga...
infection. In Chapter 4, similar strategies were used to screen and characterize candidate genes involved in rice SL biosynthesis. Intriguingly, OsCYP706C2, a homolog of ZmCYP706C37, attracted our attention. Its expression is induced by phosphate starvation and it closely co-expresses with known rice SL biosynthetic genes. Mutant analysis and chemical synthesis allowed us to identify a new rice SL, 4-oxo-MeCLA and show that OsCYP706C2 is required for its biosynthesis. Using heterologous expression in Nicotiana benthamiana and yeast, I further elucidate the biosynthetic pathway of 4-oxo-MeCLA, in which 4-oxo-19-hydroxy-carlactone is an intermediate. Moreover, bioassays using Striga and AM fungi indicate that oscyp706c2 mutants were not affected in Striga germination inducing activity but did have decreased AM fungi colonization. Taken together, in Chapter 3 and 4, I show how intricate SL biosynthesis (in maize and rice) is and shed further light on their biological significance.

In the past decades, an increasing number of natural SLs have been identified in a range of plant species. However, the low production of natural SLs hampers their identification, discovery of new biosynthetic genes and our further understanding of their biological roles and agricultural applications. Thus, exploring suitable heterologous expression systems may contribute to addressing those issues and provide opportunities for better utilization of SLs. Nicotiana benthamiana has been widely and increasingly used for transient expression of plant natural product biosynthetic pathways. In Chapter 5, I established methods to increase the SL production, through transient expression, in Nicotiana benthamiana. Several β-carotene pathway genes/gene combinations were co-expressed with the carlactone pathway genes (OsD27, OsCCD7 and OsCCD8) to investigate their boosting activity in carlactone production. Among the tested constructs, an Arabidopsis PSY-GGPS11 fusion and Zea mays ZmPSY1 showed capability in boosting the metabolic flux towards β-carotene and increased carlactone production. The possibility to further improve the flux by RNAi silencing of endogenous competing pathways of carlactone was also investigated (NbLCYE, NbCHYB), although it did not further increase carlactone level. To take this to the next level, I showed that coexpression of Arabidopsis PSY-GGPS11 and ZmPSY1 can also increase the heterologous production of two natural SLs, orobanchol and zealactone, up to 2-3 fold. This provides us with a new tool for the characterization of unknown strigolactone biosynthetic genes and possibly the production of reference standards.

Finally, in Chapter 6 I summarize the main findings of my thesis and discuss several aspects of SL biosynthesis, including the importance of SL structural diversification under selection pressure and how to control parasitic plants through modification of SL biosynthesis. Finally, I present an outlook on future research and remaining scientific challenges.
SUMMARY
Samenvatting
Strigolactonen (SLn) zijn een belangrijke klasse van plantaaardige signaalmoleculen met veel structurele variatie en diverse biologische rollen in en ook buiten de plant, in de rhizosfeer. In Hoofdstuk 1 introduceer ik de SLn, en bespreek ik hun ontdekking en biologische functies, de regulatie van hun productie door beschikbaarheid van nutriënten, en hun perceptie en downstream signalering. Ik benadruk in het bijzonder de biosynthese van SLn, inclusief die van carlacton, en de structurele diversificatie van de SLn in de biosynthetische route na carlacton. Bij dit proces van structurele diversificatie van SLn is een reeks enzymen betrokken, zoals cytochrome P450s, waarvan velen nog onbekend zijn. In mijn proefschrift bestudeer ik mogelijke benaderingen voor de ontdekking van biosynthese genen van SLn en de karakterisering van hun functie.

Parasitaire planten zoals bremraap en Striga maken gebruik van een haustorium om verbinding te maken met het vaatweefsel van hun gastheerplant, waardoor ze water, assimilaten en voedingsstoffen opnemen. Aangezien deze parasitaire planten obligate parasieten zijn, die voor hun overleven volledig afhankelijk zijn van een gastheer, moeten zij hun levenscyclus zorgvuldig afstemmen op die van hun gastheer. Daartoe hebben parasitaire planten een aantal mechanismen ontwikkeld om de gastheer te detecteren en op de gastheer te reageren. In hoofdstuk 2 worden de kiemstimulerende stoffen van de Orobanchaceae, een belangrijke parasitaire plantenfamilie, besproken, waarvan SLn de belangrijkste klasse vormen. We bespreken hoe deze verbindingen worden geproduceerd en in welke waardeplanten. En we bespreken waarom ze betrouwbare signalen zijn, hoe parasitaire planten mechanismen hebben ontwikkeld om ze te detecteren en erop te reageren, en of ze een rol spelen in gastheerspecificiteit. Inzicht in deze signalrelatie tussen gastheer en parasitaire plant verbetert ons begrip van de evolutie van plantenparasitisme en vergemakkelijkt de ontwikkeling van effectievere bestrijdingsmaatregelen wanneer parasitaire planten een onkruid vormen.

In hoofdstuk 3 en 4 heb ik de biosynthetische route en biologische functies van maïs- en rijst SLn opgehelderd. Maïs (Zea mays) is een van de belangrijkste gewassen in de wereld. In Afrika wordt de opbrengst ervan echter ernstig in gevaar gebracht door de parasitaire heksenkruidsoorten Striga hermonthica en Striga asiatica. Het exudaat van maïswortels bevat ten minste zes SLn, maar slechts twee daarvan waren structureel geïdentificeerd toen ik met mijn PhD project begon. De identiteit van de andere maïs SLn, evenals hun rol in Striga kieming en hun biosynthetische oorsprong, waren onbekend. In hoofdstuk 3 heb ik, door gebruik te maken van een combinatie van benaderingen, waaronder co-expressie analyse en (transiente) genexpressie in Nicotiana benthamiana en gist, natuurlijke variatie in de maïs SL productie aangetoond, drie nieuwe maïs SLn geïdentificeerd, en de biosynthetische route van de maïs SLn opgehelderd. Ik ontdekte dat een aantal van de biosynthese genen zich bevindt in een cluster en identificeerde een nieuw cytochrome P450, ZmCYP706C37, dat verschillende stappen katalyseert in de
biosynthese van de mais SLn. Van deze SLn hadden zealactol en zealachtonzuur een veel lagere activiteit dan zealachton, bij het induceren van Striga kieming. Ik heb ook laten zien dat veranderingen in de SL samenstelling van het wortexudaat in sommige genotypes correspondeert met verschillen in Striga kieming, en, als gevolg daarvan, Striga infectie.

In Hoofdstuk 4 heb ik vergelijkbare strategieën gebruikt om kandidaat genen die betrokken zijn bij de SL biosynthese in rijst te screenen en te karakteriseren. In dat werk trok OsCYP706C2, een homoloog van ZmCYP706C37, onze aandacht. De expressie van dit gen wordt geïnduceerd door fosfaattekort en het vertoont co-expressie met bekende genen voor de biosynthese van SLn in rijst. Mutantanalyse en chemische synthese stelden ons in staat een nieuw rijst SL, 4-oxo-MeCLA, te identificeren en aan te tonen dat OsCYP706C2 vereist is voor de biosynthese ervan. Met behulp van heterologe expressie in Nicotiana benthamiana en gist heb ik de biosyntheseweg van 4-oxo-MeCLA verder opgehelderd, waarbij 4-oxo-19-hydroxy-carlacton een tussenproduct is. Bioassays met Striga en AM schimmels lieten zien dat oscyp706c2 mutanten niet minder kieming van Striga induceren, maar wel minder efficient gekoloniseerd worden door AM schimmels. Samenvattend laat ik in hoofdstuk 3 en 4 zien hoe ingewikkeld de SL biosynthese (in maïs en rijst) is en werp verder licht op hun biologische betekenis.

In de afgelopen decennia is een toenemend aantal natuurlijke SLn geïdentificeerd in een reeks plantensoorten. De lage productie van natuurlijke SLn belemmert hun identificatie, de ontdekking van nieuwe biosynthesegeenen en ons verder inzicht in hun biologische rol en landbouwtoepassingen. Het onderzoeken van geschikte heterologe expressiesystemen kan bijdragen aan het oplossen van deze problemen en mogelijkheden bieden voor het gebruik van SLn in de landbouw. Nicotiana benthamiana wordt op grote schaal en in toenemende mate gebruikt voor transiënte expressie van biosynthese routes van plantaaardige natuurlijke producten. In hoofdstuk 5 heb ik methoden ontwikkeld om de heterologe productie van SLn, door middel van transiënte expressie in Nicotiana benthamiana, te verhogen. Verschillende β-caroteen pathway genen/gen combinaties werden samen met de carlacton pathway genen (OsD27, OsCCD7 en OsCCD8) tot expressie gebracht om hun effect op de carlacton productie te onderzoeken. Van de geteste constructen toonden een Arabidopsis PSY-GGPS11 fusie en Zea mays ZmPSY1 het vermogen om de metabolische flux naar β-caroteen te verhogen en daarmee de productie van carlacton te verhogen. De mogelijkheid om de flux verder te verbeteren door RNAi silencing van endogene concurrerende pathways van carlacton werd ook onderzocht (NbLCYE, NbCHYB), maar had geen positief effect op de carlacton productie. Om dit onderzoek naar een hoger niveau te tillen, toonde ik aan dat coexpressie van Arabidopsis PSY-GGPS11 en ZmPSY1 ook de heterologe productie van twee natuurlijke SLn, orobanchol en zealachton, kan verhogen met een factor 2 tot 3. Dit geeft ons een nieuw instrument voor de karakterisering van onbekende strigolacton biosynthese genen en
mogelijk de productie van referentie standaarden.

Tenslotte geef ik in hoofdstuk 6 een samenvatting van de belangrijkste bevindingen van mijn proefschrift en bespreek ik verschillende aspecten van SL biosynthese, waaronder de mogelijke rol van selectiedruk op de SL structurele diversificatie en hoe parasitaire planten kunnen worden bestreden door modificatie van de SL biosynthese. Tenslotte geef ik een vooruitblik op toekomstig onderzoek en resterende wetenschappelijke uitdagingen.
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