Cisgenesis of a candidate gene associated with polymorphism in steroidal glycoalkaloids biosynthesis in *Solanum dulcamara* using CRISPR-Cas

Level: MSc internship
Start: asap, latest Sept 2022
Duration: 36 ec
Location: Nijmegen
Project form: Molecular work (see details below)
Supervision: Adam Anaia and Janny Peters
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We propose to transform *S. dulcamara* mono-chemotypes, with *SdGAME25*, to assess the possibility of a gain-of-function (production of the mixed-chemotype). Examples of research questions include: **1)** Do plants with a mono-chemotype contain a (non-functional) *SdGAME25* allele? **2)** If mono-chemotype plants contain a non-functional *SdGAME25* allele, could it be edited to gain-function (e.g. production of mixed chemotype) using CRISPR-Cas? **3)** Will transformation and regeneration of mono-chemotype plants with *SdGAME25* produce a mixed-chemotype plant?

We are looking for a motivated bachelor (or master) student with experience in molecular biology lab techniques. You will..

- .. explore the possibilities of a gain-of-function in bittersweet by performing gene-editing (CRISPR-Cas) or transformation (*Agrobacterium tumefaciens* / particle bombardment) and regeneration experiments in the lab. You will use general molecular laboratory techniques (e.g. cloning, PCR, etc.)
- .. gain experience conducting scientific research
- .. integrate previous results from transcriptomics, LC-MS and qPCR with results from your own project
- .. communicate your work verbally in a presentation and written in a report

Interested student are invited to contact Adam Anaia (adam.anaia@idiv.de) or Janny Peters (janny.peters@ru.nl) with a short motivation (half A4) and a concise curriculum vitae.

This work will be a collaborative effort between Molecular-Interaction-Ecology (FSU Jena, iDiv, Leipzig, Germany) and the Plant Ecology and Physiology (Radboud University Nijmegen). The work is intended to be conducted in Nijmegen, The Netherlands.

Relevant Background Information

After rooting, plants are largely immobile. Therefore, plants have to defend themselves against pathogens, herbivores and other threats. Plant specialized metabolites (PSMs) are produced to survive in an ever-changing (a)biotic environment. The nightshade family (Solanaceae) consists of many economically important species. The genus *Solanum* - to which crop-species like potato, tomato and eggplant belong - is characterized by the production of glycosylated steroidal compounds (GSCs) including steroidal glycoalkaloids (SGAs), which are cholesterol-derived [1] nitrogen-containing steroidal (C27) glycosides. SGAs are associated with plant defense against herbivores and pathogens; different mechanisms of action were proposed including its action as deterrent (saponins are soap-forming molecules). Recently, the genes responsible for the biosynthesis of SGAs have been elucidated in crop species [2,3]. GAME25, a 3β-hydroxysteroid dehydrogenase/Δ5,4 isomerase was shown to catalyze the reaction from unsaturated SGAs to saturated SGAs in potato [4,5]. This is predicted to happen in concert with a reductase from primary metabolism [4,5].

Solanum dulcamara (known as the bittersweet nightshade[*]), is a wild, woody perennial native to Eurasia that thrives in contrasting hydrological conditions, ranging from wetlands to dry coastal dunes[6]. High structural diversity of SGAs is detected in bittersweet and compounds with different saturation levels are hypothesized to exists. Intraspecific variation of SGAs in *S. dulcamara* has been associated with differences in preferences by

[*] Among many other names, which might hint into the ancient ethnobotanical/traditional uses of *Solanum dulcamara*

generalist gastropods: 1. Gastropods preferred plants with saturated SGAs over plants producing unsaturated SGAs and 2. Gastropods preferred plants with non-nitrogenous GSCs over plants producing SGAs [7]. For specialized flea beetles, the opposite trend in preference was observed in a common garden experiment [8].
Recently, a fitness-effect has been found in a common garden in which the effect of chemotype (individual-level) and phytochemical diversity (AKA 'chemodiversity') (plot-level) on fitness measures and herbivory were assessed. Plants with unsaturated SGAs ('mono' chemotype) produced more berries than plants with 'mixed' chemotypes [producing predominantly saturated SGAs, but also unsaturated SGAs (Anaia, Unpublished Results)].
Genes known for SGA biosynthesis in *Solanum* crop species have been used to query *S. dulcamara* transcriptomes for candidate-genes based on homology. The expression of a gene-of-interest (GOI), a putative *SdGAME25*, is associated with the presence or absence of saturated compounds in *S. dulcamara*.

1. Sonawane, P. D. *et al.* Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism. *Nature Plants* **3**, 16205 (2017).
2. Cárdenas, P. D. *et al.* The bitter side of the nightshades: Genomics drives discovery in Solanaceae steroidal alkaloid metabolism. *Phytochemistry* **113**, 24–32 (2015).
3. Itkin, M. Biosynthesis of Antinutritional Alkaloids in Solanaceous Crops Is Mediated by Clustered Genes. (2013) doi:10.1126/science.1240230.
4. Lee, H. J. *et al.* Identification of a 3β-Hydroxysteroid Dehydrogenase/ 3-Ketosteroid Reductase Involved in α-Tomatine Biosynthesis in Tomato. *Plant and Cell Physiology* **60**, 1304–1315 (2019).
5. Sonawane, P. D. *et al.* Short-chain dehydrogenase/reductase governs steroidal specialized metabolites structural diversity and toxicity in the genus Solanum. *Proceedings of the National Academy of Sciences of the United States of America* **115**, E5419–E5428 (2018).
6. Zhang, Q., Peters, J. L., Visser, E. J. W., de Kroon, H. & Huber, H. Hydrologically contrasting environments induce genetic but not phenotypic differentiation in Solanum dulcamara. *Journal of Ecology* **104**, 1649–1661 (2016).
7. Calf, O. W. *et al.* Gastropods and Insects Prefer Different Solanum dulcamara Chemotypes. *J Chem Ecol* **45**, 146–161 (2019).
8. Calf, O. W. *et al.* Gastropods and Insects Prefer Different Solanum dulcamara Chemotypes. *Journal of Chemical Ecology* **45**, 146–161 (2019).