Validation of a Two-Base Pair Mutation in a *SWEET* Gene of *Solanum dulcamara* via High - Resolution Melt Curve Analysis and Characterization of Mutant Wound nectar

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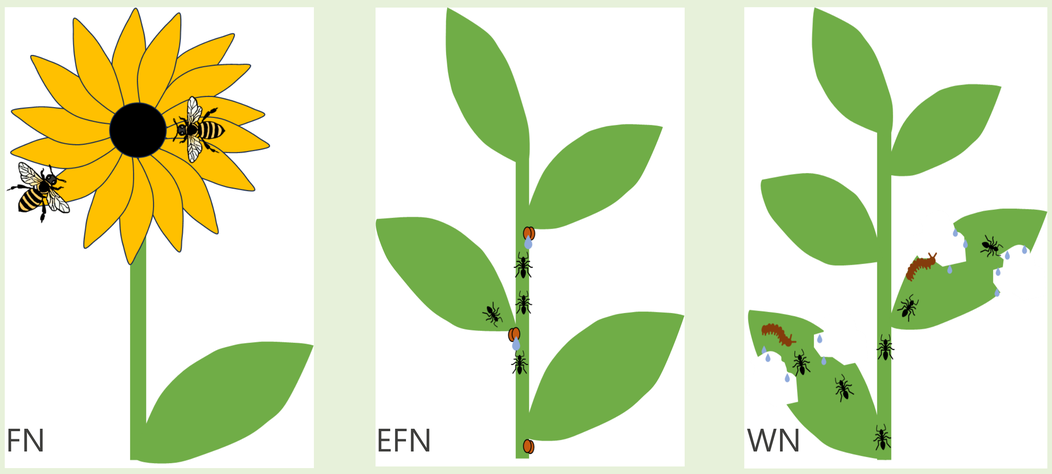
Introduction: Plants produce various forms of nectar

- Floral nectar (FN) attracts pollinators, while extrafloral nectar (EFN) serves as an indirect defense mechanism by attracting predators of herbivores, mainly ants.
- *Solanum dulcamara* produces a unique ant-attracting extrafloral nectar called wound nectar (WN) at damaged herbivore-targeted sites, independent of nectaries or specific structures.
- Investigating wound nectar release mechanisms may shed light on the evolution of nectaries, as it doesn't rely on specific nectaries or glandular tissue.
- As the nectar release from floral nectaries of several plants is known to depend on a *SWEET9* sucrose transporter, we aim to investigate whether the homolog of this gene is also involved in wound nectar secretion in *S. dulcamara*.



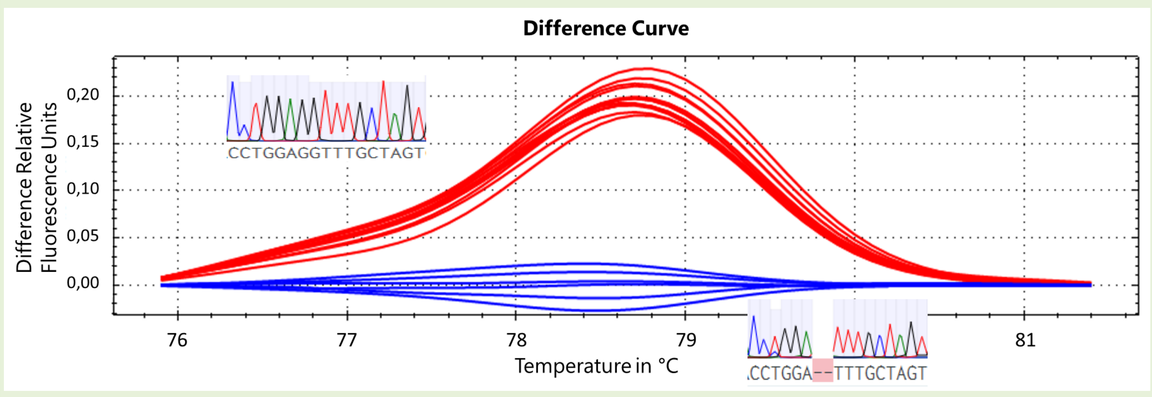
- Here we test whether High-Resolution Melt curve analysis (HRM) can be used as an easy and fast screening method to detect mutated lines among transformed plants. For this, we used CRISPR-Cas9-transformed plants for which sequencing data revealed a two-base pair mutation in the *S. dulcamara SWEET9* homolog.
- As drought can increase wound-responses in *S. dulcamara*, we compared mutated and wild type plants under well-watered and drought conditions.
- To test whether this mutation of the *SWEET9* homolog affects nectar secretion, we quantified wound nectar secretion from mutated and wild type plants.

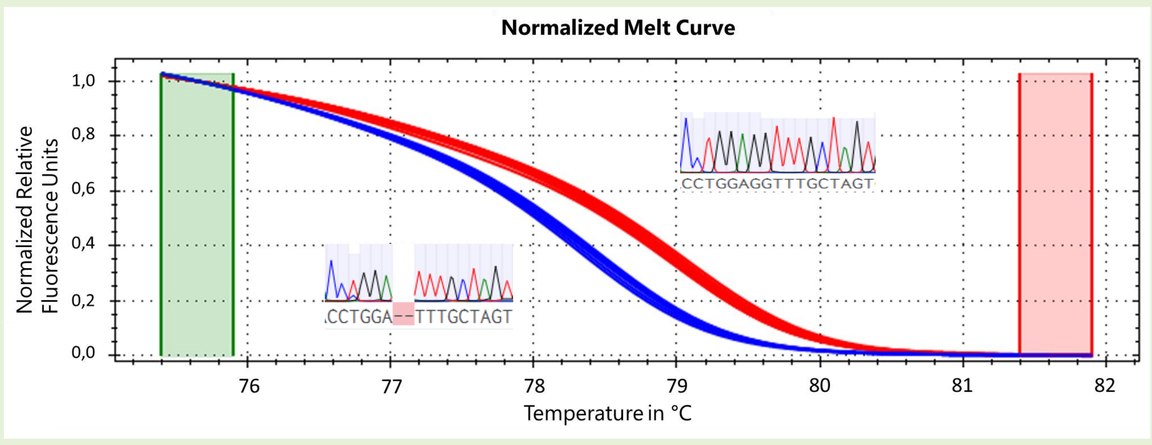
High-Resolution Melt Curve Analysis (HRM)

- HRM analysis of PCR amplicons requires a calibrated real-time PCR instrument and a specific HRM software. The high resolution is reported to allow a differentiation between amplicons of minor changes in length or nucleotide composition (down to one-base-pair-changes).
- We designed primers to target the mutation site in the *S. dulcamara SWEET9* homolog.

Result

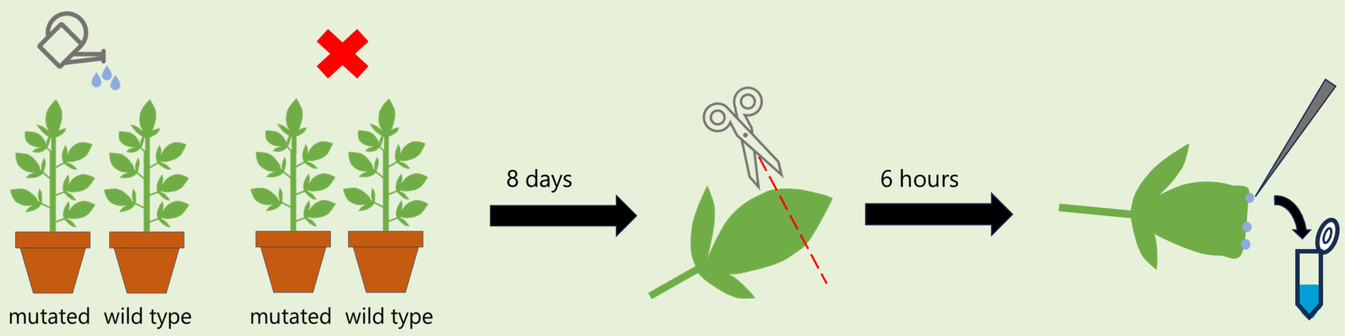
- HRM successfully differentiated between the amplicons of the *SWEET9* homolog in *S. dulcamara* generated from cDNA of wild type and mutated plants carrying a two-base-pair deletion in that gene.
- In the graph, the clustered melting curves of the wild-type plants are shown in red, while those of the mutated plants are shown in blue
- DNA's melting temperature increases with the number of G-C base pairs, as they are bonded by three hydrogen bonds instead of two. Mutated plants have two fewer G-C base pairs in their DNA, resulting in a lower melting temperature compared to the wild type.





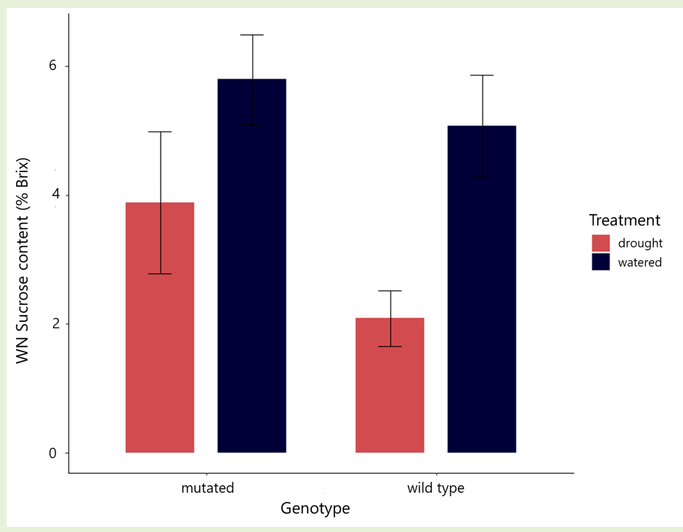
Greenhouse Experiment

- A greenhouse experiment was conducted to compare the wound nectar amount between mutant and wild-type plants.
- As SWEET9 gene expression was stronger induced by wounding in wild type plants under drought stress, we included a drought stress treatment in the experiment.
- We applied drought stress to half of both the mutants and wild-type plants for 8 days, while the other half remained under well-watered conditions.
- After 8 days, we trimmed the tips of three leaves on each plant to trigger wound nectar production. Six hours later, we gathered the wound nectar using a needle and measured the sucrose content for quantification.



Result and Conclusion

- There were no significant differences among genotypes.
- However, plants with drought stress have significantly less wound nectar comparing with well-watered plants in both genotypes. This contradicts previous findings of an increased induction under drought stress. One possible explanation maybe, that our drought treatment was stronger and resulted in a reduced photosynthesis rate. In other experiments with leaf shading, a strong dependence of the wound nectar secretion on photosynthesis was found.



- Mutant and wild type plants secrete similar amounts of sugar, suggesting the studied *SWEET9* homolog probably doesn't participate in wound nectar secretion, despite its close sequence similarity to the *SWEET9* gene responsible for floral nectar secretion in other plants.
- Further research should focus on other potential candidates. The *SWEET* gene family comprises numerous genes, of which those with an elevated expression in the wound nectar secreting tissues should be investigated. HRM can be a simple and cost-effective method to screen newly transformed plants for successful mutants of the target genes.

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