Genetic and functional characterization of carrot terpene synthases

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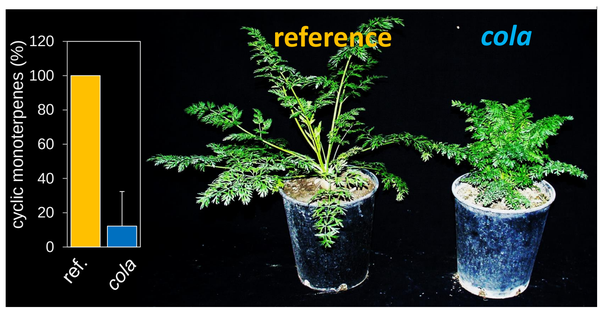
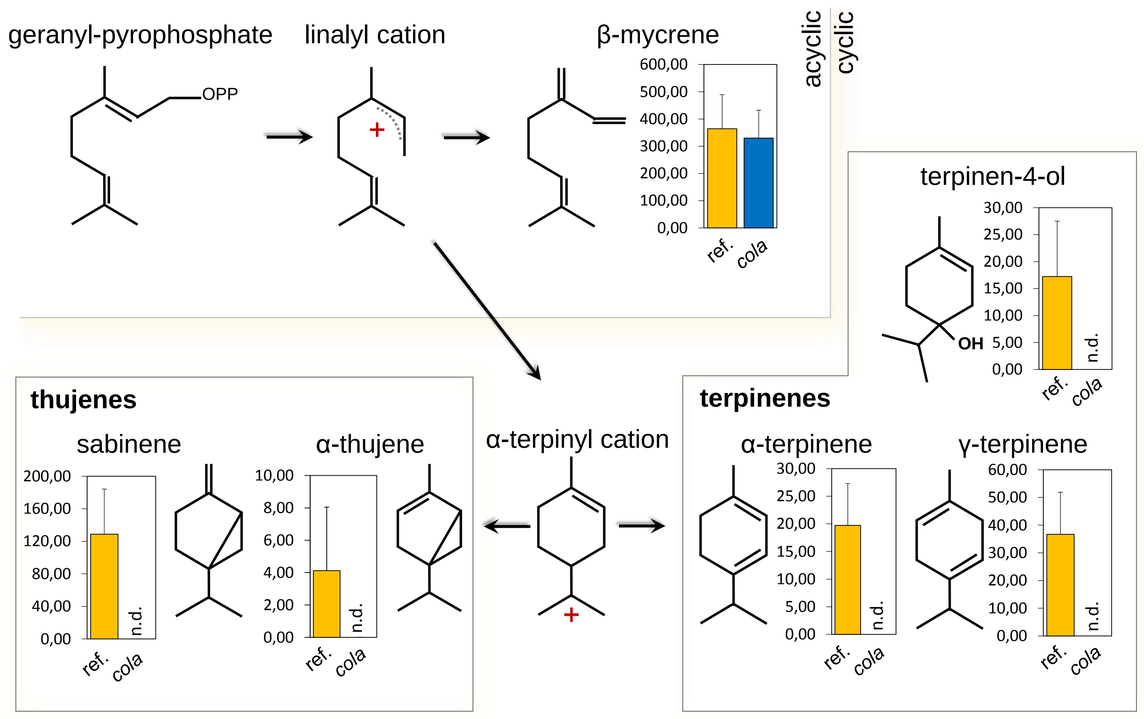
1 Cyclic monoterpene-reduced carrot mutant *cola*

Terpenes are a huge class of organic compounds with diverse physiological functions also having applications in industry and medicine. **Terpene synthases (TPSs)** are key enzymes in the formation of low molecular weight terpenes. We identified a carrot mutant that has a dwarfish habit, called *cola* (*compressed lamina*). Metabolite profiling of this mutant revealed a 87,8% decrease in the content of cyclic monoterpenes compared to the reference genotype.

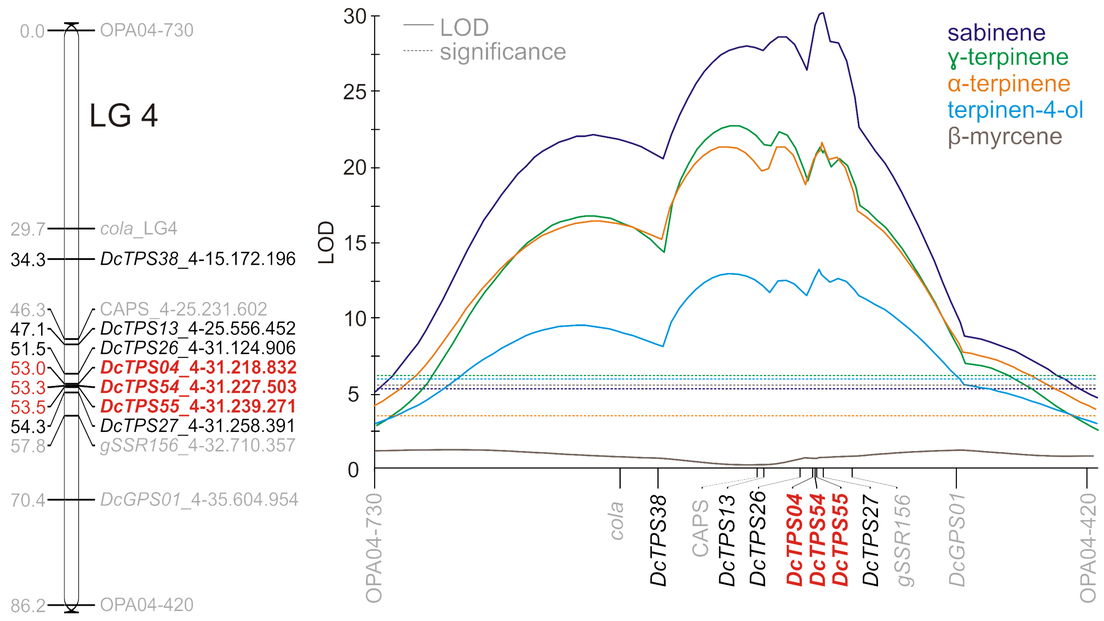




Expression of at least one TPS gene with a role in the production of thujenes and terpinenes was blocked in the *cola* mutant.

2 QTL-based identification of candidate genes

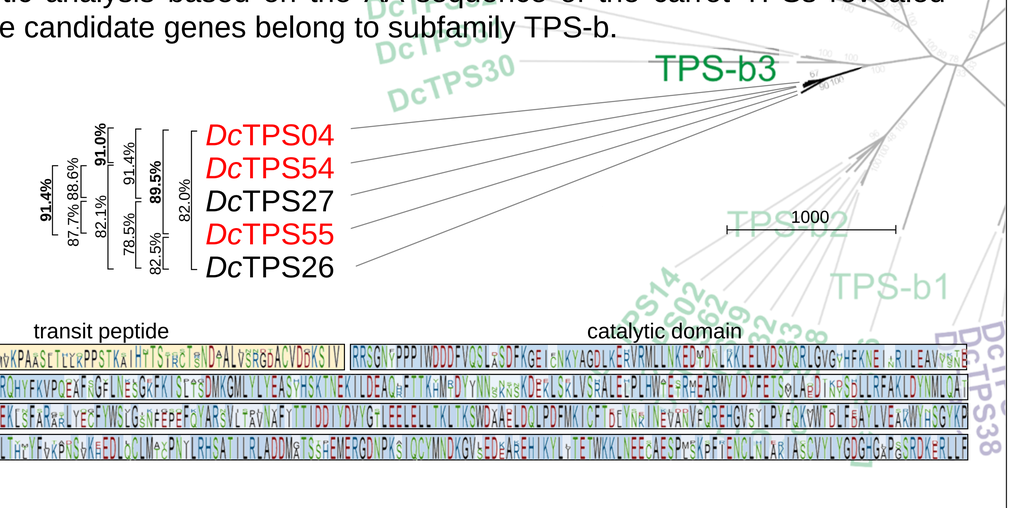
To investigate a relationship between chemical phenotype and genotype we performed a QTL analysis using a F_2-mapping population of 320 plants developed by crossing the *cola* mutant and a reference genotypes. QTLs with highly significant LOD-scores were detected for four monoterpenes in leaf material.



A gene cluster on chromosome 4 consisting of the previously annotated *DcTPS04*, *DcTPS26*, *DcTPS27*, *DcTPS54* and *DcTPS55* genes shows a highly significant correlation to biosynthesis of terpinene and thujene and their derivatives but not of β-myrcene and other acyclic monoterpenes.

3 High sequence similarity of the candidates

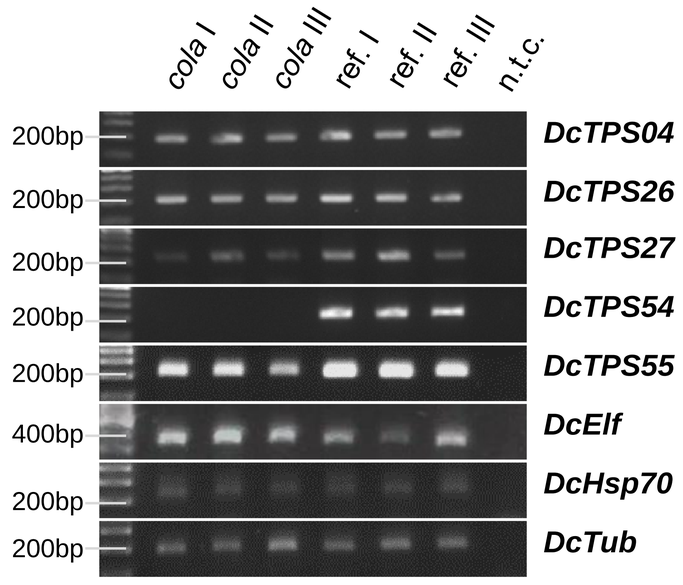
Phylogenetic analysis based on the AA sequence of the carrot TPSs revealed that the five candidate genes belong to subfamily TPS-b.



Sequence alignment showed a very high sequence similarity (~ 86 %) of the catalytic domain in the investigated candidates.

4 Expression of candidate TPSs in *cola*

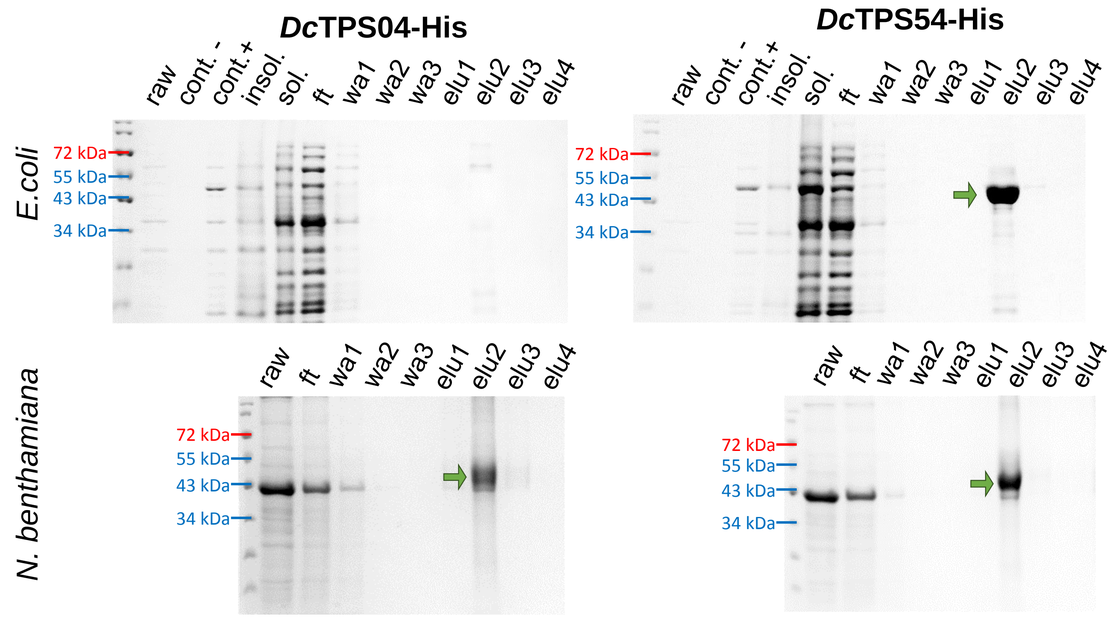
Expression of candidate TPSs was examined by semi quantitative RT-PCR in leaf material. Each sample was pooled from three independent plants.



An equal expression in the *cola* mutant and reference carrot was detected for *DcTPS04* and *DcTPS26*.
A slight reduction of *DcTPS27* and *DcTPS55* gene expression was observed in the *cola* mutant.
Interestingly there was no expression of *DcTPS54* in the *cola* mutant. We suppose that ***Dc*TPS54 might be responsible for biosynthesis of thujenes and terpinenes.**

5 Purification of recombinant TPSs

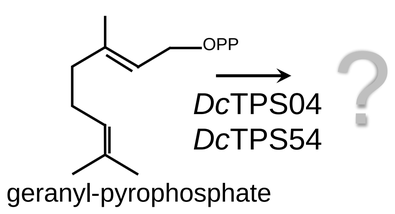
Based on the combined analysis of the obtained data we focused on cloning *DcTPS04* and *DcTPS54*. To isolate and further analyze the enzymatic activity we added C-terminal His-Tag, produced the recombinant enzymes in bacterial and plant expression systems and purified them by affinity chromatography



Expression of *Dc*TPS04 was observed in *N. benthamina* but it could not be detected in *E. coli*. *Dc*TPS54 showed a good expression level in both systems.

6 Conclusion and future prospects

We identified a cluster on chromosome 4 associated with biosynthesis of thujenes and terpinenes in carrot leaves.
This cluster includes *DcTPS04*, *DcTPS26*, *DcTPS27*, *DcTPS54* and *DcTPS55* genes, all showing a very high sequence similarity.
A combination of metabolite profiling and expression analysis gives first indication on the enzymatic activity of the TPS candidates.



Future prospects:
An *in vitro* assay might finally verify the involvement of *Dc*TPS04 and/or *Dc*TPS54 in the biosynthesis of thujenes and terpinenes.