The floral volatile phenylpropanoid/benzenoid pathway in petunia
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Plants produce a large number of secondary metabolites that aid in their development, growth and interaction with the environment. Volatile compounds mainly serve as cues in plant-animal interactions. Floral volatiles are used to attract specific pollinators, thereby facilitating pollination. The floral bouquet is generally plant-specific and in *Petunia* spp., most of these compounds are volatile phenylpropanoid/benzenoids. The volatile phenylpropanoid/benzenoid pathway is only partially characterised and produces biologically and economically important compounds. In addition to flowers, the pathway is also active in fruits and green tissues (e.g. tomato and basil). We have used *Petunia hybrida* as a model system to study biosynthesis and transport of these compounds and regulation of the pathway in plants. Closely related fragrant and non-fragrant cultivars make it a use-full system to do comparative research.

Benzoic acid (BA) is an important plant metabolite and a central compound in the (volatile) benzenoid pathway. In bacteria, the genes involved in the production of BA are cloned but in plants, they have not been characterised. Feeding experiments in plants point to the presence of a β-oxidative and a non-β-oxidative pathway. The preferred route is probably dependent on the species, tissue and environmental conditions. For the first time, we show genetic evidence for the β-oxidative pathway in plants. In **CHAPTER 2**, we identified and characterised a 3-ketocyl-CoA thiolase that catalyses the β-oxidative shortening of the carbon side chain by two C-atoms and show that in *PhKAT1*-silenced flowers, BA and benzenoid production are compromised. Our results show that in petunia flowers it is mainly the β-oxidative pathway that is active during the night, when volatiles are produced. These results argue against earlier results obtained via feeding experiments that showed higher flux through the non-β-oxidative pathway, but do not exclude the existence of this pathway in petunia petals. In addition, we showed peroxisomal localisation of PhKAT1, which indicates that the floral phenylpropanoid/benzenoid pathway is compartmentalised and is suggestive for the involvement of metabolite transporters.
Where and when genes are transcribed is largely controlled by transcription factors that bind and/or activate these genes, generally in the 5’-upstream region of the gene, where the promoter resides. The production and emission of floral volatiles in petunia is highly regulated during flower development and during the time of the day. Genes encoding biosynthetic enzymes, transporters and TFs are co-ordinately transcribed to ensure appropriate production of volatiles in the flowers. Few TFs have been described and some of their target genes have been identified, but an interaction between them has not been described. The red flowers of the non-fragrant cv. R27 do not express the R2R3-MYB TF ODORANT1 (ODO1), whereas the white flowers of the fragrant cv. Mitchell do (CHAPTER 3). We tested the activity of both ODO1 promoters in petals of both cultivars and found that it is largely the promoter that determines differences in ODO1 expression between these cultivars, rather than upstream factors. Indeed, both cultivars show small differences in the ODO1 promoter sequence. Importantly, both cultivars express another R2R3-MYB TF, EMISSION OF BENZENOID (EOBII). We show that EOBII binds and activates the Mitchell ODO1 promoter at a MYB binding site (MBS), which has a single nucleotide mutation in the R27 ODO1 promoter, rendering it inactive. A small change in the MBS is thus at the base of differential ODO1 expression between these cultivars and explains in part the lack of scent in R27 flowers, since ODO1 is necessary for fragrance production. The identification of the EOBII binding site in the ODO1 promoter will aid in the identification of more putative EOBII-target genes in silico. Our experiments further suggest combinatorial regulation at the ODO1 promoter and screening our cDNA library with the ODO1 promoter, as well as the EOBII protein, as a bait will lead to the identification of additional factors that are needed for ODO1 activation.

CHAPTER 4 describes the identification and characterisation of a petunia ABCG-transporter. The expression profile of PhABCG1 is typical for genes of the floral volatile benzenoid/phenylpropanoid pathway. Silencing does strongly to alter volatile emission. Transient ectopic expression in fragrant petunia petals does not change the emission pattern either, but results in enhanced accumulation of the volatile compound benzylbenzoate, confirming the involvement of PhABCG1 in the floral volatile
phenylpropanoid/benzenoid pathway in petunia. We further show that ODO1 regulates *PhABCG1* expression by activating the *PhABCG1* promoter *in planta*. Because *PhABCG1* is localised to the plasma membrane, intracellular transport activity can be excluded. Since we don’t know yet in which cell layer of the petal *PhABCG1* is expressed, a role in volatile emission from the epidermis or in transport of compounds between cell layers can be envisioned. Direct transport assays *in vitro* or *in vivo* should be performed to identify the substrate(s) of this transporter.