APPLE (*MALUS* X *DOMESTICA*) AS A MODEL PLANT TO INVESTIGATE THE BIOSYNTHESIS OF 3-HYDROXYPHLORIDZIN

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INTRODUCTION

Dihydrochalcones (Figure 1) are phenolic compounds which are discussed as defence mechanism against pathogens in apple [1]. However, studies on their physiological relevance are impeded by unknown biosynthetic steps like the formation of 3-hydroxyphloridzin. Polyphenol oxidases (PPO) and cytochrome P450 dependent enzymes could be involved. Although PPOs from apple have repeatedly been reported to produce 3-hydroxyphloretin as intermediates in the phloretin oxidation [2,3], their physiological relevance for the 3-hydroxyphloridzin biosynthesis in apple leaves has not yet been demonstrated. However, the involvement of such an unspecific enzyme which produces a spectrum of cell-toxic compounds in the biosynthesis of the constitutively in *Malus* sp. present 3-hydroxyphloridzin is unlikely. Hydroxylation of phloretin in position 3 has high similarity to the B-ring hydroxylation of flavonoids catalysed by the well-known flavonoid 3'-hydroxylase (F3'H). Recently we demonstrated that the dihydrochalcone phloretin is accepted as substrate *in vitro* by F3'H and the closely related chalcone 3-hydroxylase (CH3H) from the ornamental plant *Coreopsis grandiflora*. The acceptance of phloretin as substrate was also confirmed *in planta* via overexpression of *CH3H* in apple [4]. Transgenic plants showed increased contents of 3-hydroxyphloridzin and reduced susceptibility against fire blight and apple scab. We isolated *F3'H* cDNA clones from *M.* x *domestica* leaves to investigate the involvement of F3'H in the pathway leading to 3-hydroxyphloridzin.

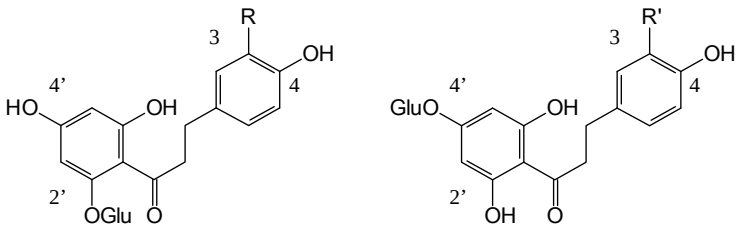

Figure 1: Main phloretin derivatives found in *Malus* species. R=H: Phloretin. R=OH: 3-Hydroxyphloretin. R'=H: Trilobatin. R'=OH: Sieboldin

EXPERIMENTS

Young leaves of *M.* x *domestica* (cv. Golden Delicious on rootstock M9) were harvested in spring 2014 in the experimental orchard of the University of Natural Resources and Life Sciences in Vienna, frozen in liquid nitrogen and stored at -80 °C. *M.* x *domestica* cv. Rebella leaves were obtained from Julius Kühn Institut Dresden-Pillnitz, Germany. mRNA was extracted with the µMACS mRNA Isolation Kit (Miltenyi Biotec, Germany). cDNA was synthetized using the SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) and an oligo-dT primer. Based on the NCBI sequence information for *F3'H* (FJ919633 and FJ919631) [5], full size *F3'H* cDNA

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clones were isolated. Heterologous expression in yeast and enzyme assays were performed as described previously [4].

RESULTS AND DISCUSSION

To investigate whether F3'H of apple could be a part of the 3-hydroxyphloridzin biosynthesis, we isolated two *F3´H* cDNA clones from apple leaves. The cDNA clones had a length of about 1.6 kb and an open reading frame of 511 amino acids. They showed sequence identity of 94% at the amino acid level to each other and of 72-74% to the F3'H of *Cosmos sulphureus* (NCBI No FJ216426). In comparison to the published sequences [5] only two exchanges at the amino acid level were observed in each of the clones. Heterologous expression in yeast resulted in functionally active F3'H only in one of the cases, despite the high sequence homology and several heterologous expression attempts. The functionally active recombinant enzyme accepted flavanones, dihydroflavonols, and flavonols as substrates. Phloretin, however, was not accepted as substrate, irrespective of the assay conditions applied. Inspection of the apple genome [6] at www.rosaceae.org did not reveal the presence of further *F3'H* copies that could be alternatively involved. Therefore, our investigations indicate that *F3'H* is not a part of the pathway leading to constitutively present 3-hydroxyphloridzin in apple leaves.

CONCLUSION

We isolated two flavonoid 3'-hydroxylase (*F3´H*) cDNAs from young leaves of *Malus* x *domestica* and heterologously expressed it in yeast. One F3´H was functionally active, but phloretin was not accepted as a substrate, thereby indicating that F3'H is not involved in the formation of 3-hydroxyphloridzin in apple leaves.

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