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Convergent evolution leading to the appearance of furanocoumarins in citrus plants.

Mariana Limones-Mendez, Audray Dugrand-Judek, Cloé Villard, Victoire Coqueret, Yann Froelicher, Frédéric Bourgaud, Alexandre Olry, Alain Hehn

1- Université de Lorraine, INRA, LAE, F54000, Nancy, France.
2- CIRAD, UMR AGAP, F-34398 Montpellier, France.
3- Plant Advanced Technologies SA, F-54500 Vandœuvre-lès-Nancy, France.

& Equal contribution

# Corresponding author: Alain.Hehn@univ-lorraine.fr

Summary:

Furanocoumarins are defense molecules mainly described in four plant families that are phylogenetically distant. Molecular characterization of the biosynthetic pathway has been started for many years in Apiaceae and Rutaceae. The results obtained thus far in Apiaceae indicated a major role of cytochromes P450 (P450s) in the CYP71 family. In the present work, we describe the importance of another subfamily of P450s, CYP82D, identified by using a deep analysis of the citrus (Rutaceae) genome and microarray database. CYP82D64 is able to hydroxylate xanthotoxin to generate 5-OH-xanthotoxin. Minor and limited amino acid changes in the CYP82D64 coding sequence between Citrus paradisi and Citrus hystrix provide the enzyme in the latter with the ability to hydroxylate herniarin, but with low
efficiency. The kinetic constants of the enzyme are consistent with those of other enzymes of this type in plants and indicate that it may be the physiological substrate. The activity of the enzyme is identical to that of CYP71AZ6 identified in parsnip, showing possible evolutionary convergence between these two families of plants. It is highly possible that these molecules are derived from the synthesis of ubiquitous coumarins throughout the plant kingdom.

Keywords: Cytochrome P450, CYP82, Furanocoumarins, *Citrus*, Convergent evolution

Author contributions:
MLM and ADJ did the cloning and functional characterization of the enzymes. ADJ and VC did the qPCR experiments and metabolic analysis. AO did the enzymatic characterization. CV produced the phylogenetic tree. YF, FB and AH supervised the project. AH wrote the article.

Introduction
Furanocoumarins (FCs) are specialized metabolites that have been widely reported as being involved in the defense of plants against aggressors [1]. These molecules, although described in higher plants, are mainly distributed in 4 families: Apiaceae, Moraceae, Fabaceae and Rutaceae [2,3]. Two main kinds of FCs exist depending on the position of the furan ring on the coumarin core molecule. The tracks leading to either linear or angular molecules are catalyzed by enzymes belonging to the UbiA Prenyltransferases. A prenylation occurring on C6 leads to the synthesis of the linear demethylsuberosin whereas a prenylation on C8 gives ostheno (Figure 1) [4–6] The presence of both isomers are, thus far, only reported in Apiaceae and are reported to reflect a coevolutionary process between plants and caterpillars [7]. These four plant families are quite distant from a phylogenetic point of view, and the
independent appearance of these molecules remains unexplained at the molecular level. The first enzymes involved in the synthesis of these molecules to be characterized were a psoralen synthase (CYP71AJ1-3) and an angelicin synthase (CYP71AJ4) [8,9]. Both enzymes belong to the cytochrome 450 enzyme superfamily. CYP71AJ4, identified in *Pastinaca sativa*, may result from the duplication of CYP71AJ3 followed by neofunctionalization [8]. This assumption is consistent with the high amino acid sequence identity shared by the two proteins (69.8 %) and is reinforced by the results published by Roselli et al., who showed their clustering in the parsnip genome (the genes are 7.6 kb apart) [10]. Relatively minor changes in the peptidic sequence of psoralen synthase therefore allowed the appearance of the angular FC angelicin and subsequent angular molecules, thus participating in the arms race between plants and insects. An extensive study of this specific CYP71AJ subfamily has led to the identification of numerous orthologous genes, but their function could not be demonstrated [11].

Krieger et al have highlighted another cytochrome P450 subfamily, CYP71AZ, involved in the synthesis of FC [12]. CYP71AZ4 is able to hydroxylate psoralen, leading to the synthesis of xanthotoxol, whereas CYP71AZ1 and CYP71AZ6 catalyze the hydroxylation of xanthotoxin, generating 5-OH-xanthotoxin (Figure 1). Interestingly, CYP71AZ6 seems quite specific to a single molecule (xanthotoxin), while CYP71AZ4 can transform different types of molecules, including a FC (psoralen) and coumarins (6-methoxycoumarin, scopoletin, 7-methoxy-3-methylcoumarin, and 7-methoxycoumarin) [12]. Finally, Krieger et al also showed that CYP71AZ3 seems to be specific and dedicated to the hydroxylation of esculetin, another coumarin [12]. All higher plants are able to produce coumarins and the fact that some members of the CYP71AJ and CYP71AZ families are involved in the synthesis of coumarins and/or FCs [11,12] provides new insights for understanding the evolutionary processes leading to the appearance of FCs that might have diverged from coumarins. To date, the
different enzymes described to be involved in the synthesis of FCs belong to the same large
CYP71 cytochrome P450 family. This family is responsible for the synthesis of highly
diversified and specialized metabolites. For example, members of the family are involved in
the synthesis of artemisin [13], menthofuran [14], camalexin [15,16], cyanogenic glucosides
[17] or DIMBOA [18]. The diversity of chemical structures, biosynthetic pathways and plant
species make this enzyme family a major actor in the adaptation of plants to their
environments.

The CYP71 family is not the only cytochrome P450 that seems to be involved in the synthesis
of such molecules (Figure S5). The functional characterization of CYP82C2 and CYP82C4
isolated from Arabidopsis thaliana reinforces the assumption that FCs are derived from
coumarins. Kruse et al showed that these enzymes convert in vivo xanthotoxin to 5-OH-
xanthotoxin when overexpressing Arabidopsis mutants are incubated in a culture media
containing the substrate (Figure 1) [19], while this plant is not otherwise able to synthesize
this kind of molecule. In 2018, Rajniak et al. showed that CYP82C4 is also involved in the
oxidation of fraxetin (a coumarin), leading to the synthesis of sideretin [20], which is
described as being important in iron homeostasis [21]. Taken together, these results indicate
that the functionalization of coumarins into FCs may be realized in different ways (CYP71 or
CYP82) throughout higher plants, thus supporting the convergent evolution hypothesis.

Rutaceae are reported to produce coumarins and linear FCs [22–24], but less is known about
the enzymes involved in this synthesis. However, a cytochrome P450 associated with an
evolutionary process related to the synthesis of FC was described. Gravot et al. showed that
the cinnamate 4-hydroxylase (C4H) of Ruta graveolens was adapted to the presence of FCs.
Although inhibited by psoralen and xanthotoxin through mechanism-based inactivation, the
C4H isolated from R. graveolens displayed a lower sensitivity to these molecules than the
C4H of Helianthus tuberosus [25]. Citruses belong to the Rutaceae family. The consumption
of these fruits has a wide range of beneficial effects for human health, since they are rich in vitamin C, folates, alimentary fibers and minerals [26]. In addition to these positive effects, it has also been demonstrated that the presence of FCs generates deleterious effects such as phototoxicity and photosensitivity [27–30] and a drug interaction effect known as "the grapefruit juice effect" that can cause overdose in humans by inhibiting liver cytochromes P450 involved in the detoxification of xenobiotics [31–33].

In this work, we investigated the biosynthesis of FCs in citrus species, focusing on the CYP82 family. The expression pattern of four genes and their functional characterization showed that two of them perform the hydroxylation of xanthotoxin to produce 5-OH-xanthotoxin. The physiological relevance of this result is supported by a quantitative PCR approach that shows a clear correlation between gene expression and the amount of these molecules.

**Material and methods**

**Citrus data**

Citrus genomic and transcriptomic data were collected from the Phytozome project database (https://phytozome.jgi.doe.gov/) and the RNAseq database published by Voo et al [34].

**Phylogenetic analysis**

The tree was built with the maximum likelihood (PhyML) method. The alignment was performed with MUSCLE using the default settings [35]. The variable regions creating gaps, the C-terminal region and the N-terminal membrane anchor were removed. One hundred iterations were performed, and the bootstrap values were calculated. The tree was rooted with CYP71AJ3, CYP71AJ4, CYP71AZ3, CYP71AZ5 and CYP71AZ6 from *P. sativa,*
CYP71AU3, CYP71AU4 and CYP71AH4 from *Vitis vinifera*, and CYP83B1 and CYP83A1 from *A. thaliana*.

**RT-PCR and cloning strategy**

RNA was extracted from flavedo of *C. paradisi* Star Ruby and *C. hystrix* fruits using the E.Z.N.A.® Plant RNA kit following the manufacturer’s recommendations (Omega Bio-Tek). Residual genomic DNA was removed using amplification-grade DNase I (Sigma-Aldrich). Reverse transcription was performed with a High-Capacity RNA-to-cDNA Kit (Applied Biosystems). The coding sequences were amplified with high-fidelity PrimeSTAR® Max DNA polymerase (Takara) and ligated into the pCR8 plasmid using the pCR8 TA/GW/Topo kit (Invitrogen). The designed primers were as follows: CYP82D64 Fw 5' ATG GAG TTC CTA TTC ACA GCT GTA ATA ATC ACT GCC C 3', CYP82D64 Rev 5' TCA ATG ATG ATG ATG AAA GCC ATA GGG GTT GGC A 3'; CYP82D83 Fw 5' ATG GAC ATT CTT GGT TCG TCT CAA AC 3', CYP82D83 Rev 5' TCA ATG ATG ATG ATG ATG ATG GCC GTA CAA AGA AGC AGA AAG AC 3'; CYP82D92 Fw 5' ATG GAC TTC CTT GTT TTA ACA TCT CAA C 3', CYP82D92 Rev 5' TCA ATG ATG ATG ATG ATG ATG GCC GTA CAA AGA AGC AGA AAG AC 3'; and CYP82D179 Fw 5' ATG GAC TTT CTT GCC ATA CAG AGA AGC AGA GAG 3'. For each ORF, we added a 6xHIS tag before the stop codon. Finally, the ORF was transferred into the pYeDP60 GW [12] plasmid using Gateway LR Clonase II Enzyme mix (Invitrogen).

**Expression of proteins and enzymatic characterization**

The recombinant proteins were produced in a yeast expression system [36]. The culture conditions, the preparation of microsomes and the biochemical screening were as described by Krieger et al. (2018).
Reverse transcription was performed with a High-Capacity RNA-to-cDNA Kit (Applied Biosystems). The primers used were $EF1\alpha$ Fw 5'-TGG TGA TGC TGG GTT TGT TA-3', $EF1\alpha$ Rev 5'-CAC GCT CTT GAT GAC TCC AA-3' for the reference gene; $CYP82D64$ Fw (1) 5'-GGC GCC ATG CTT TCC A-3', $CYP82D64$ Rev (1) 5'-ACG ACC GGG AAA TTC TTG TG-3'; and $CYP82D64$ Fw (2) 5'-CAC CAC GTG AAG CAA TGG AA-3', $CYP82D64$ Rev (2) 5'-TGC CAG CCG GGA CAT G-3'. qPCR was performed with SYBR Premix Ex Taq (Takara) based on the manufacturer’s recommendations. The amplification program was 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 34 sec. The PCR was followed by a single run at 95°C for 15 sec, 60°C for 60 sec, and 95°C for 15 sec in order to assess the melting curve and therefore the specificity of the PCRs.

Enzymatic characterization

All the enzymatic reactions were conducted in 0.1 M KPi buffer. pH and temperature range were set between 6.0 to 8.0 and 15 °C to 40 °C. For the kinetic determination of optimal temperature and pH, saturating concentrations of substrates were used (100 µM) in phosphate buffer. For km kinetic parameter determination, xanthotoxin concentration was set between 0.5 and 300 µM and the reaction was conducted at optimal pH and temperature. 20 µl of the yeast microsomal preparation was added to each enzymatic incubation and enzymatic reactions were running during 5 min and stopped by the addition of 10 µl HCl 1N. Substrates and the resulting products were analyzed by HPLC at a wavelength of 320 nm as described by Vialart et al. Kinetic parameters were calculated using the SigmaPlot 12.0 software. Identification of the chemical structure of the products was done by comparison with their elution time in comparison to 5-OH-xanthotoxin standard molecule and by LTQ orbitrap as described by Krieger et al. (2018)
Phytochemical analysis

Methanol extraction and phytochemical analyses were performed as previously described by Dugrand et al. [22].

Results

Identification of cytochrome P450 coding sequences belonging to the CYP82 subfamily in Citrus genomic and microarray databases.

The Phytozome database (https://phytozome.jgi.doe.gov/) contains the genomes of Citrus sinensis and Citrus clementina. These genomes were annotated [37,38], but regarding cytochromes P450, the annotation was limited to the superfamily level. According to Nelson, two P450s belong to the same family if they share more than 40% amino acid identity and are in the same subfamily if they share more than 55% (http://drnelson.utmem.edu/CytochromeP450.html). To seek candidate genes, we performed a sequence homology search using the BlastN and tBlastX algorithms and CYP82C2 and CYP82C4 from A. thaliana as bait sequences. This first investigation led us to identify 79 P450 candidate sequences that were further restricted to 42 CYP82 partial sequences in the two Citrus species (16 for C. sinensis and 26 for C. clementina). Most were redundant, which led us to finally consider only 8 different coding sequences (Table 1). All the sequences were affiliated with the CYP82C (CYP82C46) and CYP82D (CYP82D64, 81, 82, 83, 84, 92, and 179) subfamilies according to the cytochrome P450 annotation committee (http://drnelson.utmem.edu/CytochromeP450.html).

To complete this first analysis, we compared our results with the database provided by Voo et al [34]. This analysis showed that 2 sequences of the 8 selected previously, i.e., CYP82D64 (i.e., contigs Cit.29478 and Cit.2373 in the database) and CYP82D79 (i.e., Cit.5945 in the
database), had differential expression (Table 1) between flavedo oil cavities (tissue with a high concentration of FCs, [22,23]) and albedo tissue (lack of FCs). These first analyses supported these two sequences as strong candidates involved in FC biosynthesis in *Citrus* spp.

To obtain a more detailed overview of the distribution of this P450 subfamily, we performed a phylogenetic analysis including 91 members of the CYP82 subfamily available in databases in the literature and various other databases (Supplemental figure 1). The tree was rooted with 11 sequences belonging to the CYP71 or CYP83 subfamily. This analysis showed that the already-characterized CYP82C2 and CYP82C4 were located on different branches than the CYP82D identified in citrus plants (Figure 2). Within the more specific citrus clade, we could not distinguish different groups that could provide clues for functional characterization since the genes were distributed between *C. sinensis* (unable to produce FCs) and *C. clementina* (producing FCs) [23].

**Functional characterization of the CYP82D candidates.**

In our analysis, CYP82D64, CYP82D83, and CYP82D179 were detected in both plants, whereas CYP82D82 and CYP82D81 were specific to *C. sinensis* and CYP82D92 was found only in *C. clementina*. Since the aim of our study was to identify genes involved in the synthesis of FCs, we focused our attention on CYP82D92, CYP82D64, CYP82D83, and CYP82D179, detected either in *C. clementina* alone or in both plants. The study performed by Krieger et al showed that minor modification of the sequence of CYP71AZ can lead to significant changes in substrate specificity and/or enzyme activity. To limit the risk of cloning gene variants displaying low FC biosynthetic activities, we amplified and cloned the cDNA coding sequences from *C. paradisi*, a citrus species producing diverse and large amounts of FCs [22,23]. The amplified sequences of *C. paradisi* showed minor changes (98 to 100%
identity) in comparison to the genomic sequences of *C. sinensis* or *C. clementina*. These differences were, however, not located in the enzyme active sites [39].

To identify their function, the 4 His-tagged P450s were expressed in a yeast heterologous expression system, and their expression was assessed by Western blot (Supplemental figure 2). CYP82D64, CYP82D92 and CYP82D179 were efficiently produced and further incubated in the presence of NADPH and the different intermediates of the biosynthetic pathway (demethylsuberosin, marmesin, xanthotoxol, xanthotoxin, bergaptol, bergapten, 5-OH-xanthotoxin, 8-OH bergapten and isopimpinellin) (Figure 1). Only metabolization of xanthotoxin could be observed in the presence of CYP82D64 (Figure 4A). The elution time and the fragmentation spectrum (maximum fragmentation intensities for m/z of 172, 217 and 205, for a total m/z of 233) were identical to those of 5-OH-xanthotoxin (Figure 4 B-E). No enzymatic activity could be detected for any other protein tested.

**Plasticity of substrate specificity between two CYP82D64 variants isolated from *C. paradisi* and *C. hystrix***.

Several studies have provided evidence that only a few modifications to an amino acid sequence can alter the specificity of an enzyme. Concerning the synthesis of coumarins and FCs, this has been shown for CYP71AZ3, 4 and 6, which are able to transform esculetin, psoralen and xanthotoxin, respectively [12]. We cloned the CYP82D64 orthologous gene from combava (*C. hystrix*) fruits, a citrus species known to be a very high producer of FCs [23]. The CDS that we obtained from this plant was named CYP82D64v2 and showed several differences compared with the *C. paradisi* coding sequence (CYP82D64v1), especially in the substrate recognition sites SRS3, 4 and 6, which were reported to be important for the enzymatic activity of cytochromes P450 [39] (Figure 4). Microsomes prepared from yeast
overexpressing CYP82D64v2 were able to convert xanthotoxin into 5-OH xanthoxine at the same rate as CYP82D64v1. Surprisingly, CYP82D64v2 was also able to transform herniarin (7-methoxycoumarin) at a rather low rate into a product that was identified as 5-hydroxy-7-methoxycoumarin (Figure 5).

Enzymatic characterization of CYP82D64v1 and v2

The optimal pH of both CYP82D64s was assessed in phosphate buffer with a pH ranging from 6.0 to 8.0. Under these conditions, we showed that the hydroxylation activity of xanthotoxin was effective over the entire pH range and was optimal at a pH of 7.3 (Supplemental figure 3). The activity in the presence of herniarin could not be detected below a pH of 6.4, but the optimal activity was obtained at a pH of 7.2. The optimal temperature of the hydroxylation of both molecules was close to 28°C (27.8°C for xanthotoxin and 28.1°C for herniarin) (Supplemental figure 4). Finally, the kinetic constants (km) of CYP82D64v1 and v2 were determined to be 4.6+/-1.7 µM and 7.4+/-4.0 µM for xanthotoxin, respectively. These km values were rather low and consistent with those reported in studies performed on other cytochromes P450. This specificity may reflect a physiological activity. Because of the too-low enzymatic activity, the km could not be determined for herniarin.

In vivo expression of CYP82D64

Although the km of the reaction was consistent with a physiological function, we wanted to assess if there was a relationship between the expression level of the mRNA coding for CYP82D64 and the amounts of xanthotoxin and 5-OH-xanthotoxin produced in C. paradisi fruit at different stages of maturity: young, intermediate and mature (Figure 6A). We observed that the expression level of CYP82D64 mRNA significantly decreased during fruit
development (Figure 6B). It was reduced 2.3-fold between the young and intermediate fruits and 18-fold between the intermediate-stage and ripe fruits. Regarding the expression of CYP82D64 mRNA, the concentrations of xanthotoxin and 5-OH-xanthotoxin also decreased significantly. The average content of the 2 molecules decreased 2.5-fold between the maturation of young fruits to the intermediate stage and 7.4-fold during the transformation from intermediate-stage to ripe fruits.

Discussion

The Rutaceae and Apiaceae families are quite distant in terms of angiosperm evolution. Interestingly, however, they are both able to produce FCs [23,24], although the diversity of molecules differs between them. Apiaceae, including parsnip, are able to synthesize both linear (i.e., psoralen derivatives) and angular isomers (i.e., angelicin derivatives) [8]. Rutaceae and, in particular, the Citrus genus produce only a linear isomer [23]. The first step involved in the production of these molecules concerns the C2'-hydroxylation of coumaroyl-CoA, leading to the synthesis of umbelliferone [10,40,41]. Umbelliferone is commonly described in many higher plants. This step is catalyzed by 2-oxoglutarate-dependent dioxygenase in R. graveolens [40] and P. sativa [10], which belong to the same DIOX30 clade [41], and therefore seems to be well conserved across angiosperms. The prenylation of umbelliferone has not yet been described in citrus but was reported for Apiaceae plants and is catalyzed by a UbiA prenyltransferase [4]. A recent study by Munakata and colleagues showed that in Ficus carica, a Moraceae able to produce FCs, prenyltransferase had a different evolutionary origin than the enzyme involved in the synthesis of demethylsuberosin in Apiaceae. It might therefore be possible that convergent evolution occurred at this stage [42].
Studies based on biochemical approaches performed in the 1980s pointed out that in Apiaceae, many enzymatic steps happening after the synthesis of demethylsuberosin (Figure 1) were catalyzed by enzymes belonging to the cytochrome P450 superfamily [43,44]. These results were confirmed by the identification of the CYP71AJ [8,9] and CYP71AZ subfamilies [12], which are involved in the synthesis of psoralen, angelicin, xanthotoxol and 5-OH-xanthotoxin. To identify the genes involved in the same reaction in other plant species, we searched for CYP71AJ or CYP71AZ orthologous genes in citrus databases. Since this investigation was unsuccessful, we extended our investigation to other cytochrome P450 families. Based on the work performed by Kruse and colleagues [19], who described the hydroxylation of 8-methoxypsoralen (i.e., xanthotoxin) by a P450 isolated from Arabidopsis, we focused on the CYP82 family. Among several candidate genes, we characterized CYP82D64 as a xanthotoxin hydroxylase. This enzyme displayed the same enzymatic function as CYP71AZ6 isolated from parsnip [12]. The low km and the close relationship between the expression level of the gene and the accumulation of 5-OH-xanthotoxin suggest that this reaction also occurs in planta as a physiological reaction.

In this study, we provided evidence that CYP82D64 was able to hydroxylate herniarin. This result is in line with that from a study recently published by Rajniak supporting the role of CYP82C4 in the production of sideretin, another coumarin [20]. This report, in addition to our results, highlights that the CYP82 family participates in the synthesis of both FCs and coumarins, as does the CYP71AZ subfamily in Apiaceae. These elements provide new insights in favor of the evolution of the FC biosynthetic pathway from the coumarin biosynthetic pathway. These results are also consistent with the extreme diversity of the CYP71 and CYP82 subfamilies (53 CYP71s and 25 CYP82s reported to date), demonstrating the plasticity of these enzymes that seem to be deeply involved in the adaptation of plants to their environments. Indeed, similar to the CYP71 subfamily, CYP82 members are associated
with highly functionally diverse families of molecules with very different chemical structures such as alkaloids, terpenes or phenolic compounds. For example, CYP82N3 and CYP82N4 enzymes of *Papaver somniferum* hydroxylate alkaline benzylisoquinolines [45]. CYP82E4 from *Nicotiana tabacum* corresponds to a nicotine N-demethylase [46]. CYP82G1 of *A. thaliana* is involved in the synthesis of volatile homoterpenes [47]. CYP82D63 from *Mentha piperita* and CYP82D33 from * Ocimum basilicum* hydroxylate flavones [48]. CYP82D64 from *C. paradisi* and CYP82C2 from *A. thaliana* hydroxylate xanthotoxin, and CYP82C4 hydroxylates fraxetin. This functional diversity was distributed over the entire generated phylogenetic tree (Figure 2).

In this work, we provide some new lines of evidence in favor of a convergent evolution theory for explaining the presence of FCs in Rutaceae and Apiaceae. It is highly possible that the same process occurred in Moraceae and Fabaceae, two other plant families producing large amounts of these specialized molecules. It is therefore not excluded that other P450 families or subfamilies play an important role in the synthesis of FCs and the subsequent adaptation of these plants to their environments. Such convergent evolution is not unique in the plant kingdom. Genes encoding enzymes belonging to different families may have identical functions, such as the anthocyanin-5-O-glucosyltransferases of *Dianthus caryophyllus* and *A. thaliana*. In this case, the enzymes have different structures but share the same glycosylation of anthocyanin function [49]. Similarly, ferulate-5-hydroxylase (CYP788A1) from *Selaginella moellendorffii* shares only 37% identity with angiosperm P450s but plays the same role as these P450s in the synthesis of S units of lignin [50].

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References


The Distribution of Coumarins and Furanocoumarins in Citrus Species Closely Matches Citrus Phylogeny and Reflects the Organization of Biosynthetic Pathways., Plos One. 10 (2016) e0142757 DOI: 10.1371/journal.pone.0142757.


Cloning and characterization of a C4H from a psoralen producing plant - Ruta...


[45] G.A.W. Beaudoin, P.J. Facchini, Isolation and characterization of a cDNA encoding (S)-cis-N-methylstylopine 14-hydroxylase from opium poppy, a key enzyme in sanguinarine


Figures:

Figure 1: schematic representation of the furanocoumarin biosynthetic pathway and the enzymatic steps catalyzed by cytochrome P450s.

Red arrows: known steps; gray arrows: P450 putative steps; black arrows: catalyzed by other enzyme families.
Figure 2: phylogenetic tree of the CYP82 family.


Figure 3: enzymatic conversion of xanthotoxin by CYP82D64.

A) DAAD chromatogram, B) UV spectrum of the product, C) MS analysis of the product, D) MS analysis of commercial 5-OH-xanthotoxin, E) UV spectrum of commercial 5-OH-xanthotoxin.
Figure 4: sequence comparison of CYP82D64 variants isolated from *C. clementina*, *C. paradisi* and *C. hystrix*.

Red stars highlight the differences, and green stars show the substrate recognition sites [39]. The *C. clementina* sequence was predicted from the CDS available in Phytozome.

Figure 5: enzymatic conversion of herniarin by CYP82D64v2.

A) DAAD chromatogram, B) UV spectrum of the product, C) MS analysis of the product, D) MS analysis of commercial 5-hydroxy-7-methoxycoumarin, E) UV spectrum of commercial 5-hydroxy-7-methoxycoumarin.

Figure 6: comparison of the expression level of *CYP82D64* mRNA to the xanthotoxin and 5-OH-xanthotoxin contents in *C. paradisi* fruits. A) Three stages of maturity were tested: immature, intermediate and mature fruits. B) Expression level of CYP82D64 and concentrations of xanthotoxin and 5-OH-xanthotoxin.

**Supplemental Data**

Supplemental figure 1: list of the P450 sequences used to create the phylogenetic tree in Figure 2

Supplemental figure 2: Western blot analysis of microsomes prepared from yeast expressing CYP82D64, CYP82D83, CYP82D92 and CYP82D179. Void: yeast transformed with empty pYeDP60 plasmid; Positive: yeast expressing CYP71AZ4 (Krieger et al, 2018).
Supplemental figure 3: optimal pH determination for CYP82D64v1 and v2
A) Optimal pH for CYP82D64v1 with xanthotoxin, B) optimal pH for CYP82D64v2 with xanthotoxin, C) optimal pH for CYP82D64v2 with herniarin.

Supplemental figure 4: optimal temperature determination for CYP82D64v1 and v2
A) Optimal temperature for CYP82D64v1 with xanthotoxin, B) optimal temperature for CYP82D64v2 with xanthotoxin, C) optimal temperature for CYP82D64v2 with herniarin.

Supplemental figure 5: Phylogenetic tree constructed with the predicted cytochrome P450s belonging to the Clan71 of *Citrus sinensis* and *Citrus clementina*. The tree has been rooted with CYP51G1. Sequence alignment was performed using MAFFT relying on Multiple sequence alignment default settings (https://mafft.cbrc.jp/alignment/software). The tree was buildt with IQ-TREE multicore version 1.6.11 for Linux 64-bit built Jun 6 2019 and FigTree.
Main figures
Figure 1:
Figure 2

- **CYP82N**: Protopine 6-hydroxylase
- **CYP82H**: N-methylstylopine 14-hydroxylase
- **CYP82C**: Xanthotoxin-5-monooxygenase / Fraxetin-5-hydroxylase
- **CYP82F**: Xanthotoxin-5-monooxygenase
- **CYP82A**: Flavone-6-hydroxylase
- **CYP82D**: Xanthotoxine-5-monooxygenase
- **Citrus clade**
- **CYP82B**: 
- **CYP82E**: Nicotine N-demethylase
- **CYP82S**: Homoterpene biosynthesis
- **CYP82G**:
Figure 4
Figure 5
Figure 6

A

B

C

5 cm

B

Pulp

Concentration (mg/g Fresh weight)

Relative density of CYP83D4

- Xanthotoxine, p < 0.05
- 5-hydroxy-xanthotoxine, p < 0.05

Maturity stage

Immature

Intermediary

Mature
Table 1

<table>
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> XP_006471503_(CS)
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> CYP82D29_(CP)
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> CYP82D29_(CP)
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> CYP82D179_(CP)

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> XP_006471473_(CS)

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> XP_006471496_(CS)

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> CYP82D33_AG630364_(OB)

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1159
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>CYP82D1_1(MS)
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KNVQDFIDVLSSLHGYDVDTIKACLTL'
LHAGATDDTHTITTWALSSLNLNNRLHLLKVKQDELDEKGVKDRLNVESDINNLVYLA
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54
Supplemental Figure 2
Supplemental figure 4
Supplemental figure 5