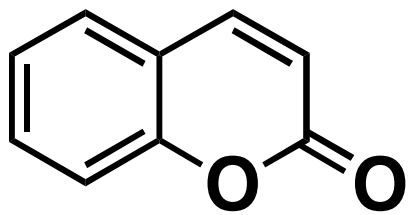
ストレスに応答する二次代謝産物生合成とその利用の可能性



Coumarin

清水文一

植物の防御反応と防御物質

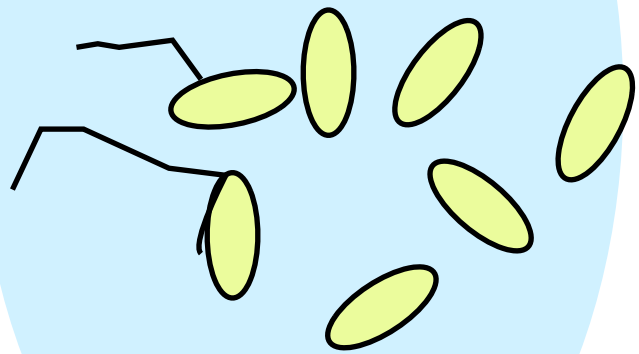
植物

Secondary Metabolites
Cell-Wall Degrading Enzymes
Enzyme Inhibitors

侵入者

Fungus, Bacteria, Insects...

Toxins
Degrading Ezymes



Chemical Battle

兵器の研究

～無駄なものは作っていないはず!

二次代謝と植物防疫

育種などで選抜されている ＞＞ あまり積極的に利用されていない?

<u>生合成経路に関する情報</u>

遺伝子資源としての利用

・・・・ 組み換え体　抗ストレス植物の作出

薬剤の作用点としての利用

・・・・ 一次代謝だけでなく二次代謝も

薬剤のターゲットとして利用できないか?

二次代謝産物の多様性

生物種によって固有のものが多い

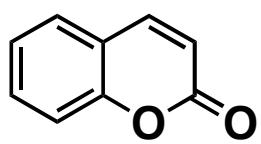
・・・・薬剤の選択性

Coumarins

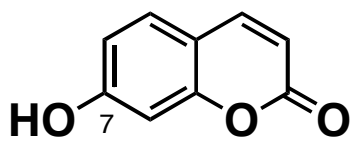
Activities --

- **Antibacterial**
- **Antifungal**
- **Antioxidative**
- **Allelopathic**

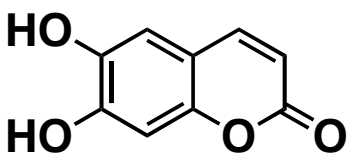
ex.)



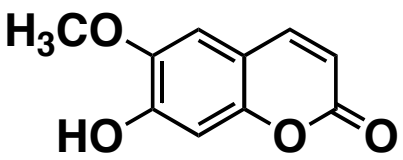
Coumarin



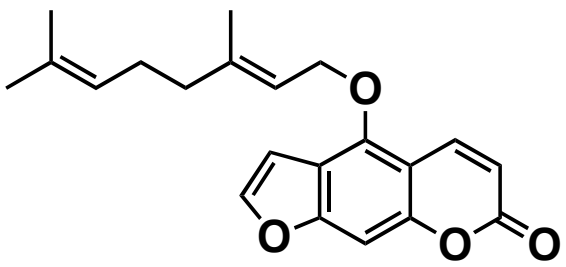
Umbelliferone



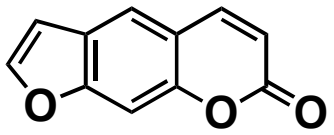
Esculetin



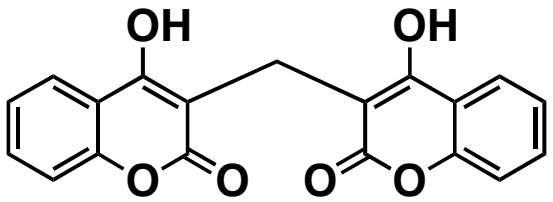
Scopoletin



Bergamotin



Psoraren



Dicoumarol

Induced Resistance in Sweet Potato

Sweet potato in the infested field by *Fusarium* wilt
(caused by pathogenic *Fusarium*: **PF**)

No Treated

Non-Pathogenic *Fusarium* (**NPF**)

K. Ogawa, *Bull. Nat. Agric. Res. Cent.,* **10**, 1988

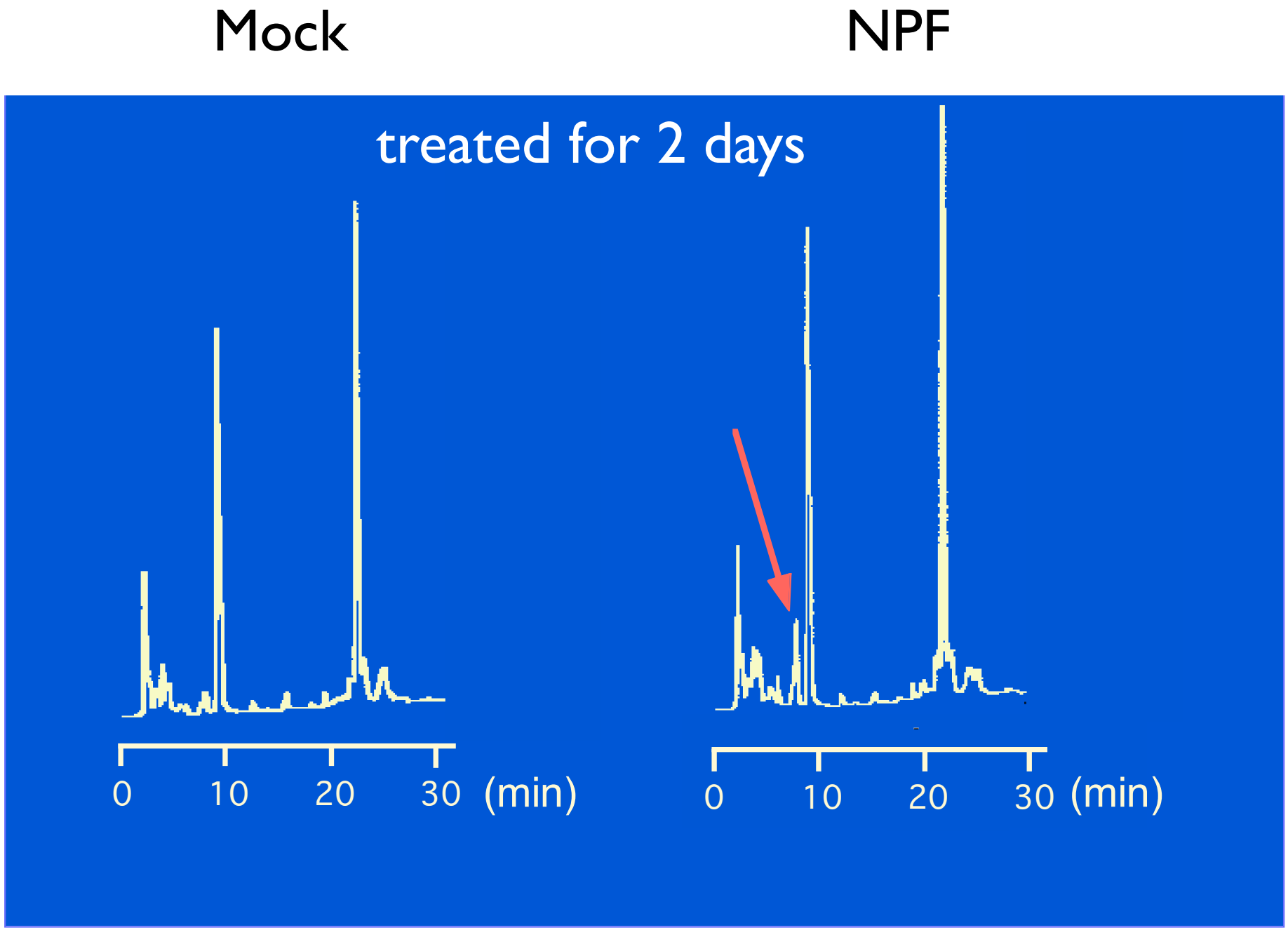
Resistance in Morning Glory Induced by Non-pathogenic *Fusarium*

Symptom of Wilt-Disease
(10 days treatment with PF)

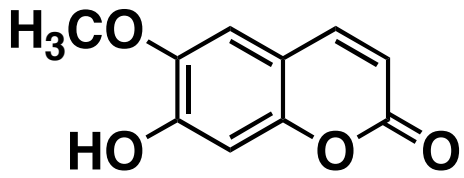
Non-pathogenic
Fusarium

Mock

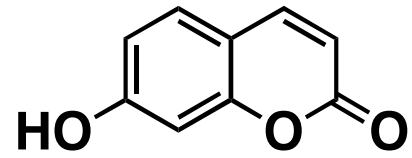
HPLC analysis of the stem extracts (UV 280nm)



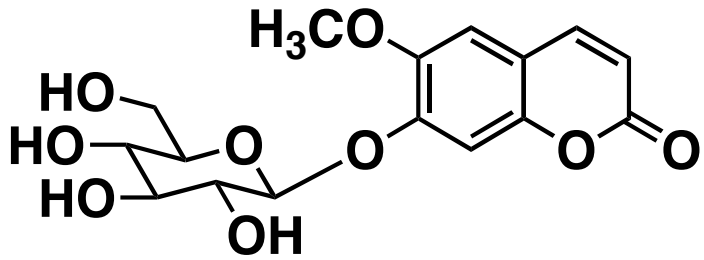
Identification of the induced components



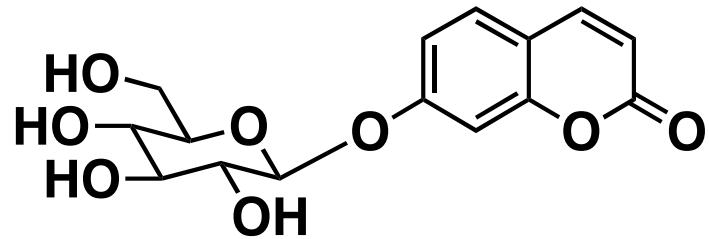
Scopoletin



Umbelliferone



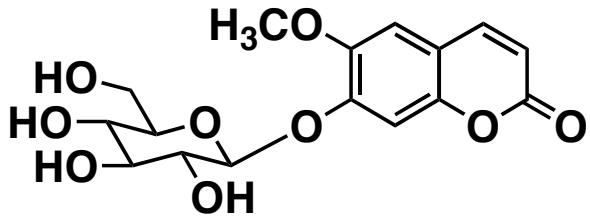
Scopolin



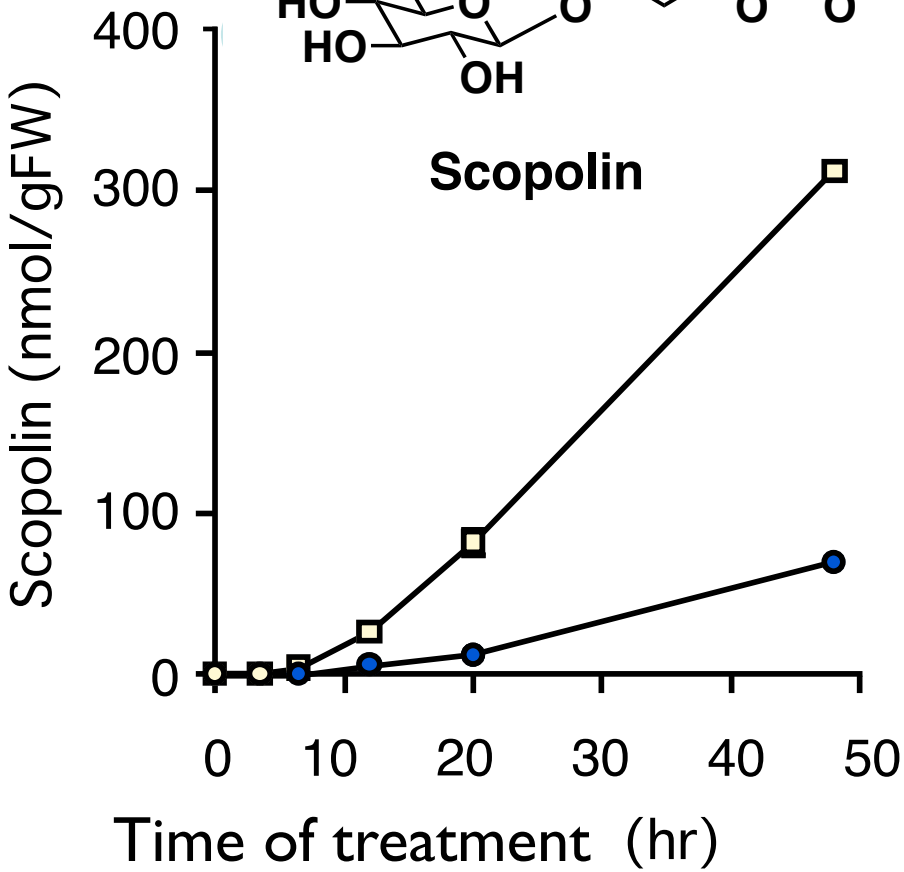
Skimmin

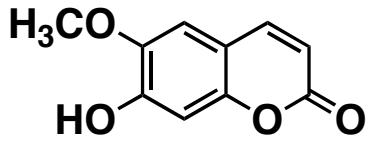
by NMR, LC/MS

Scopoletin/scopolin induction in morning glory

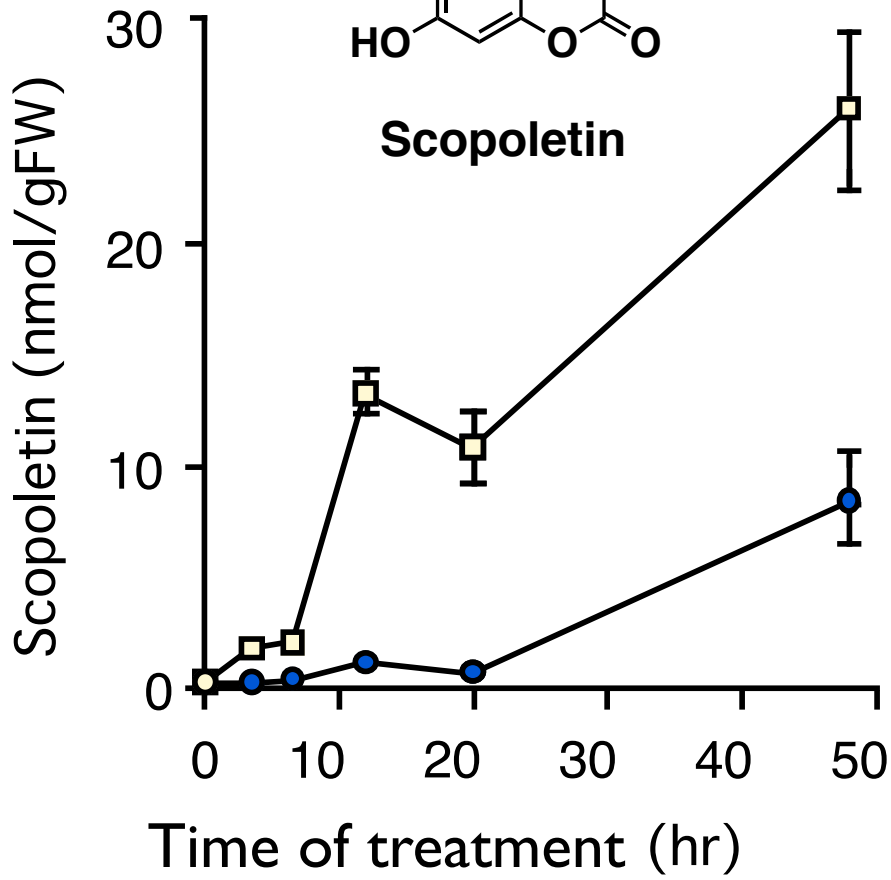


Scopolin





Scopoletin

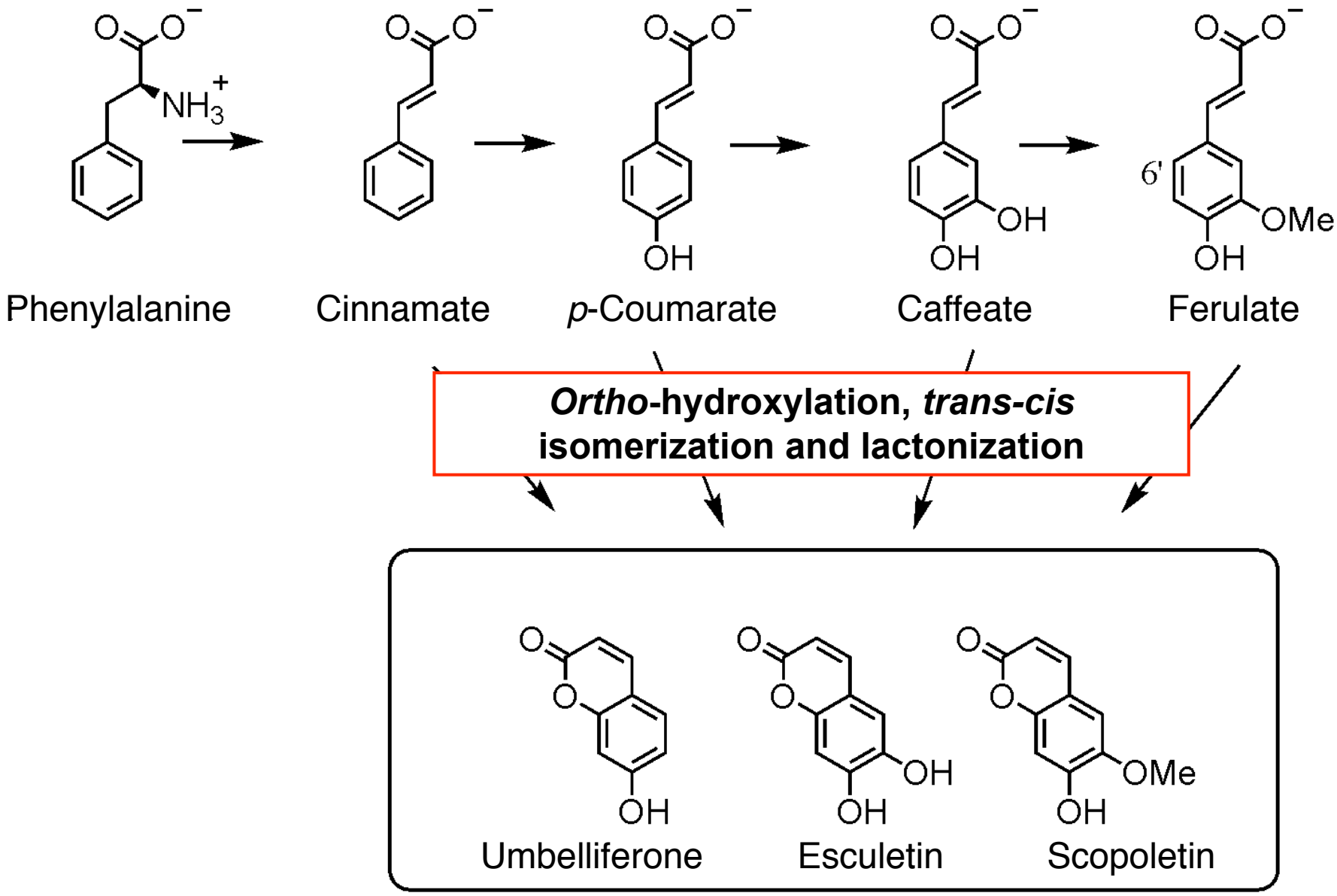


Mock

NPF (10^8 bud-cell/ml)

bar: SE (n=3)

Phenylpropanoid pathway and coumarin biosynthesis



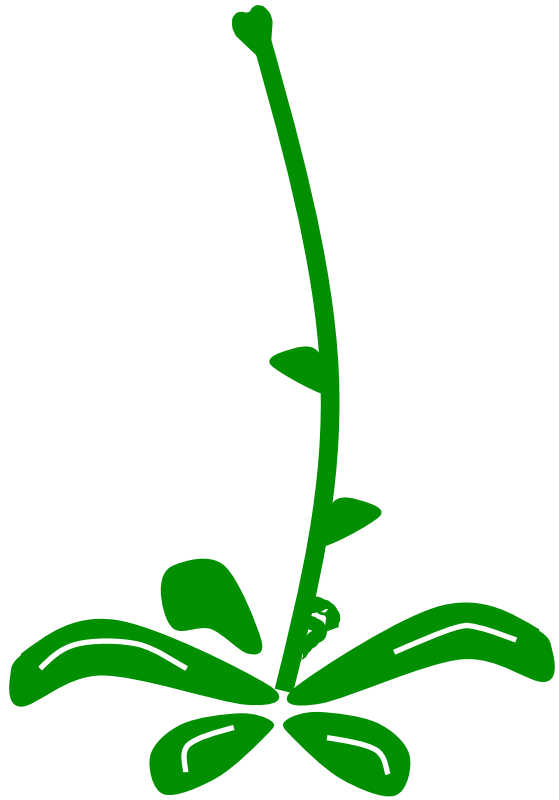
Arabidopsis thaliana, the model plant

because:

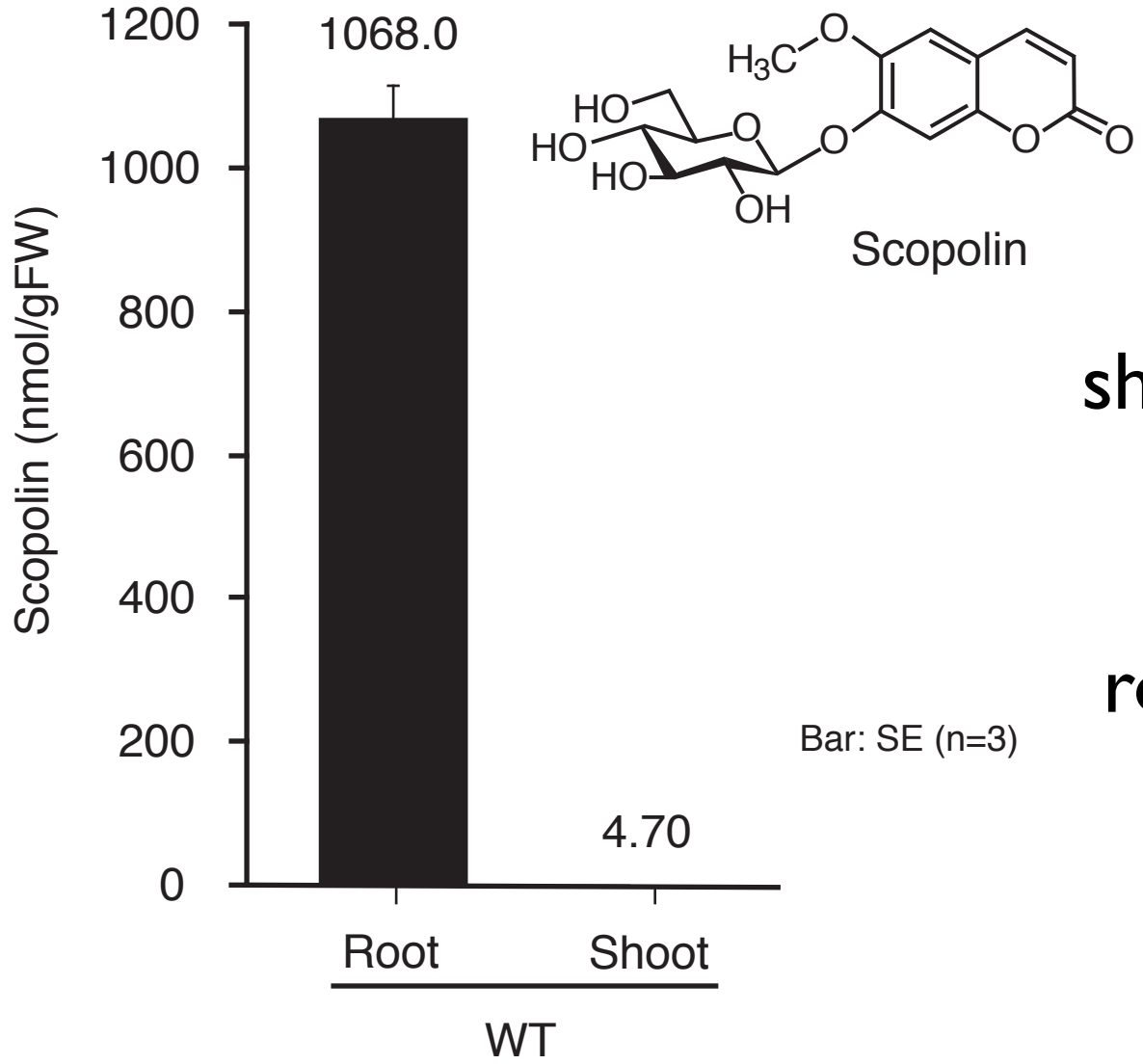
1) reproduction cycle is short

2) easy to grow in labo.

3) good facilities
(genome information, data bases, mutant libraries)

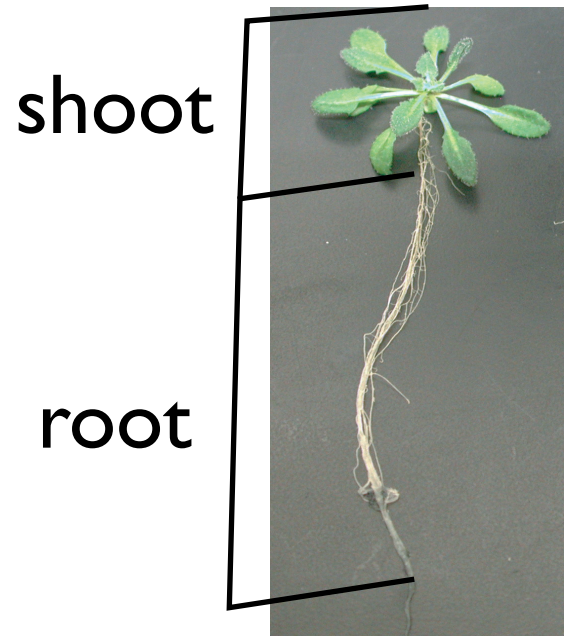


Scopoletin accumulation in Arabidopsis



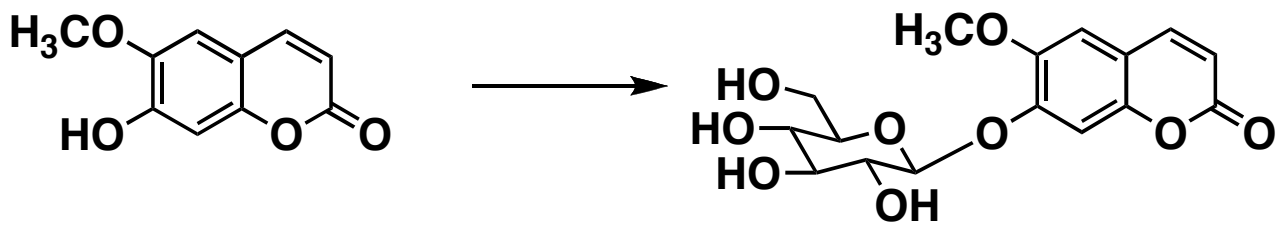
Scopolin

Bar: SE (n=3)

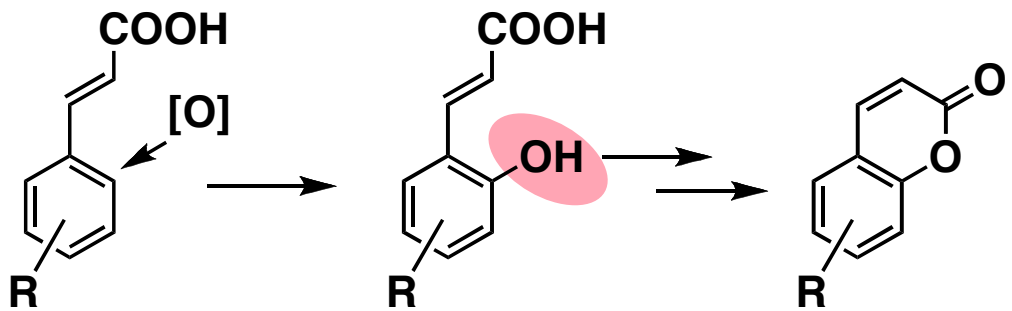


Enzymes involved in scopolin biosynthesis

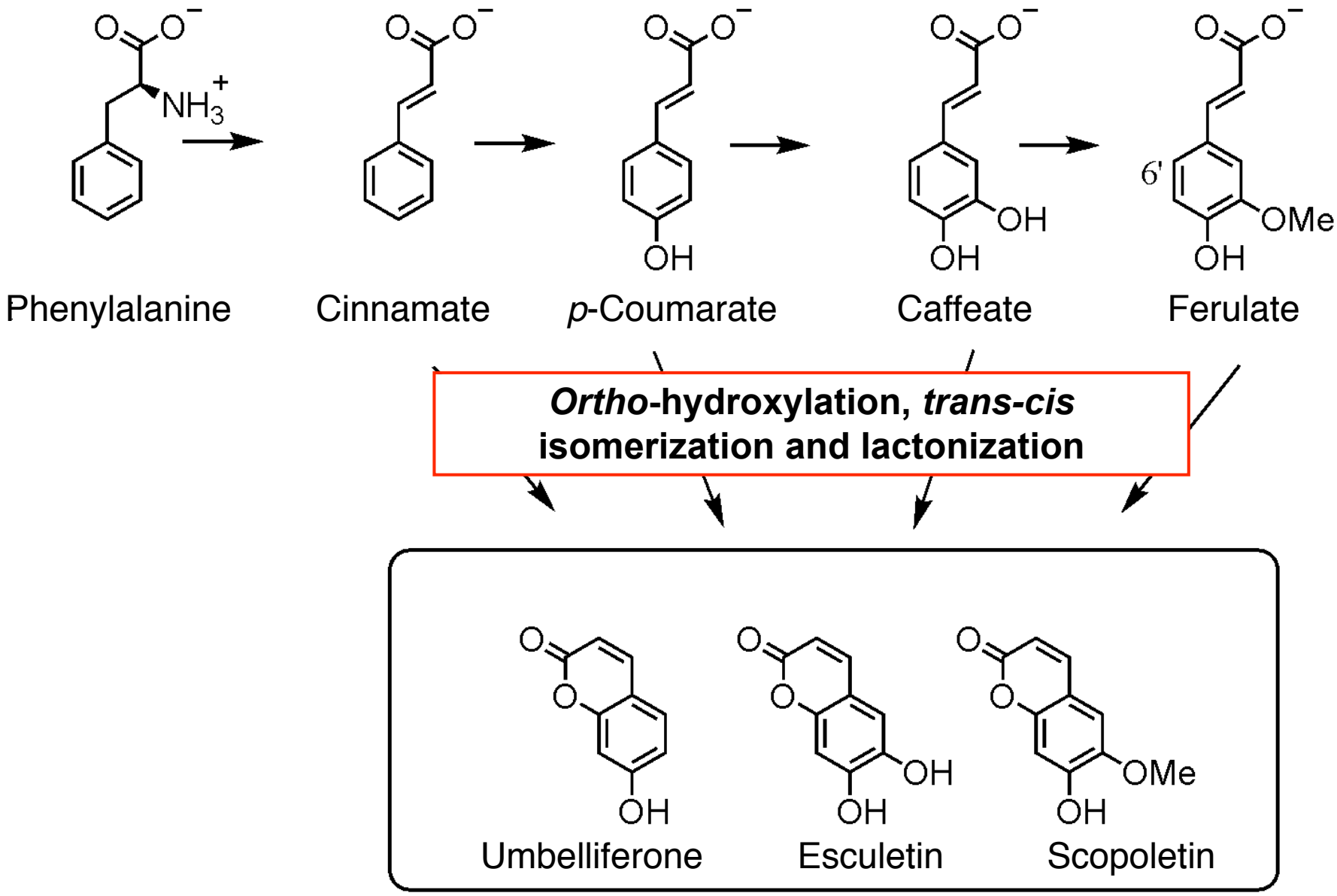
1. **Glucosyltransferase**



2. **2'(*ortho*)-Hydroxylation**

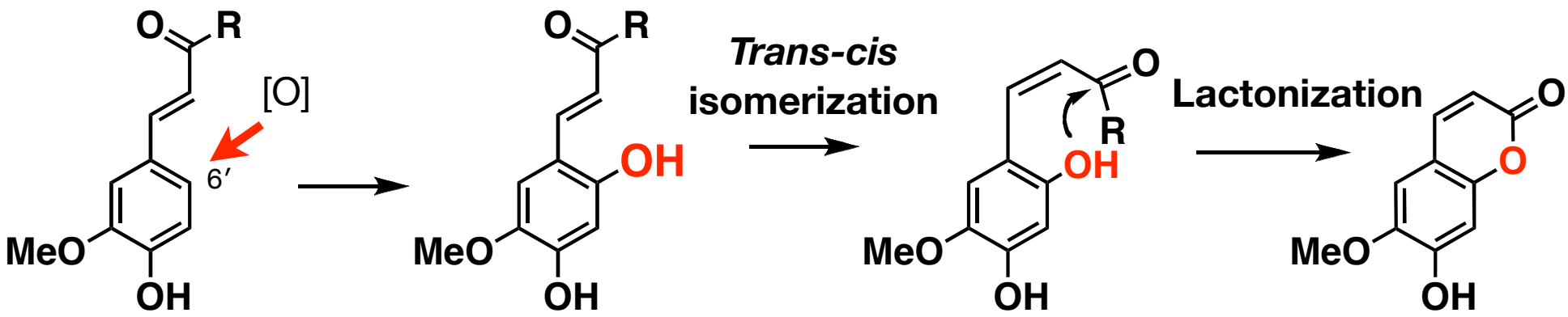


Phenylpropanoid pathway and coumarin biosynthesis

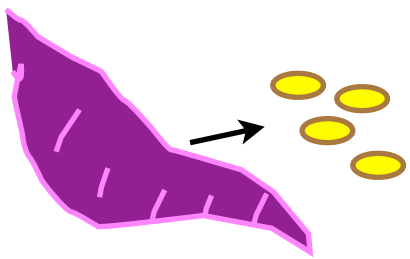


Ferulate 6′-hydroxylase (F6′H)

6′-Hydroxylation

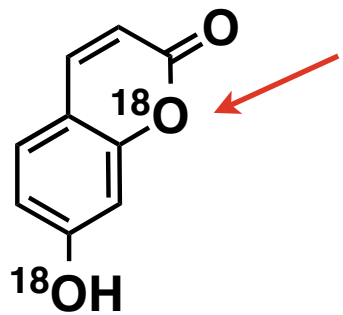


Tracer analysis using $^{18}O_2$



Root discs of sweetpotato

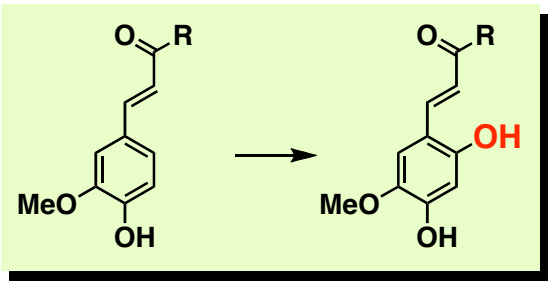
$^{18}O_2$



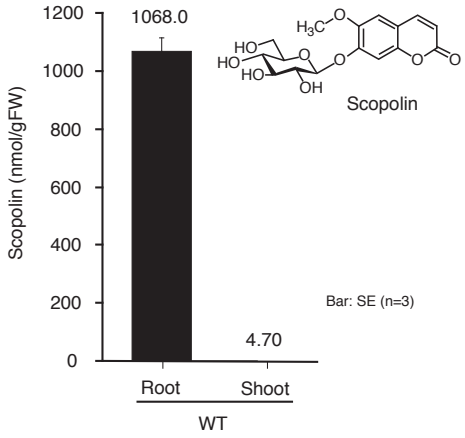
LC/MS/MS analysis

The *ortho*-hydroxylase in coumarin formation uses MOLECULAR OXYGEN.

F6′H attributes



- √ Expressed in roots （roots > shoots）.
- √ Induced by 2,4-D treatment .
- √ Uses molecular oxygen



  - Cytochrome P450 monooxygenase
  - **2-Oxoglutarate-dependent dioxygenase**
  - Flavin monooxygenase

→ Candidate genes are selected using databases, MPSS (http://mpss.udel.edu/at)

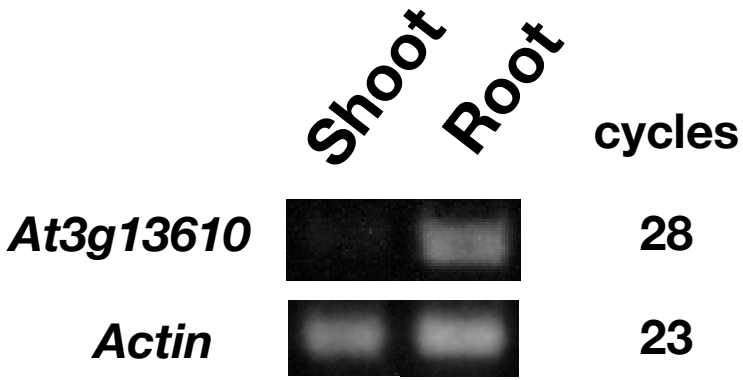
Selection of the candidate of F6'H

Candidate genes from ATTED*	Enzyme type	mpss signiture information**			
		Inflorescence	Leaf	Root	Silique
At4g13770	Cytochrome P450 monooxygenase	0	118	29	0
At1g06640	2-Oxoglutarate dependent dioxygenase	28	43	112	33
At2g17720	2-Oxoglutarate dependent dioxygenase	150	134	419	86
√At3g13610	2-Oxoglutarate dependent dioxygenase	6	12	261	3

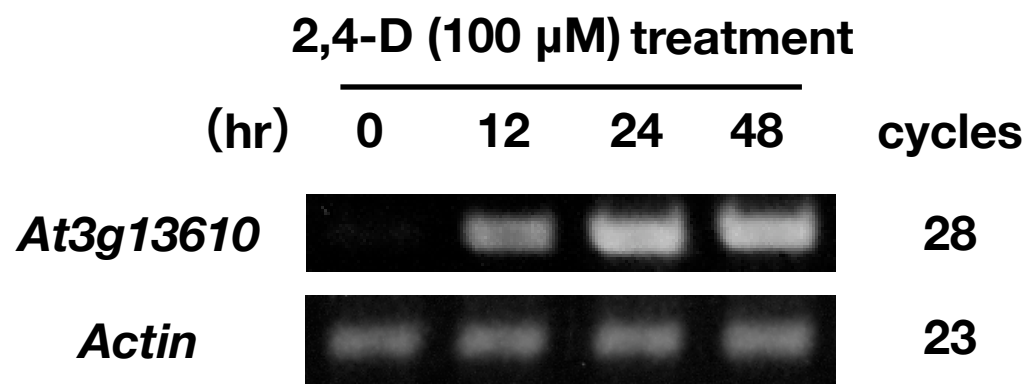
* http://www.atted.bio.titech.ac.jp, ** http://mpss.udel.edu/at

Expression pattern of the At3g13610 gene

Comparison of roots and shoots



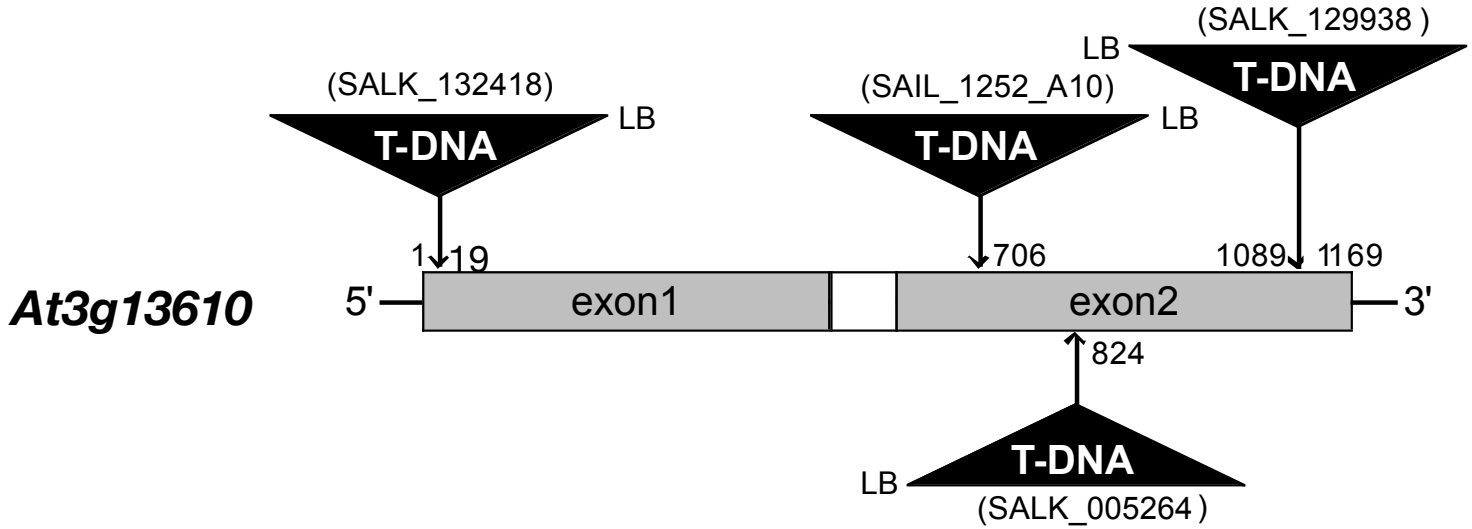
Response against 2,4-D in shoots



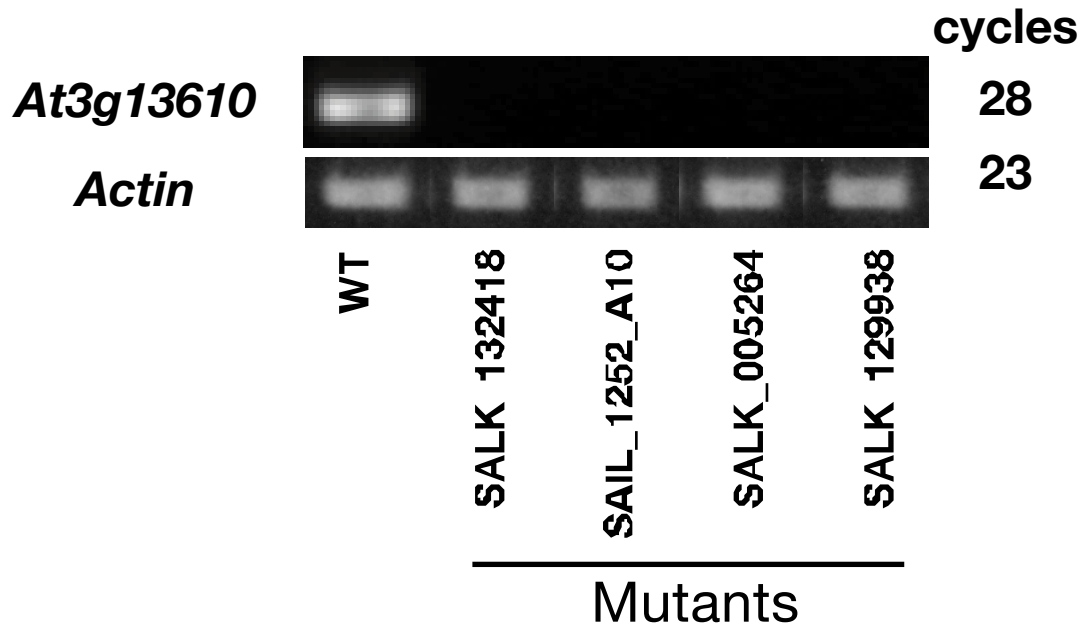
2,4-D: 2,4-Dichlorophenoxyacetic acid

Plant Journal, **55**, 989-999, 2008

T-DNA inserted mutants of At3g13610



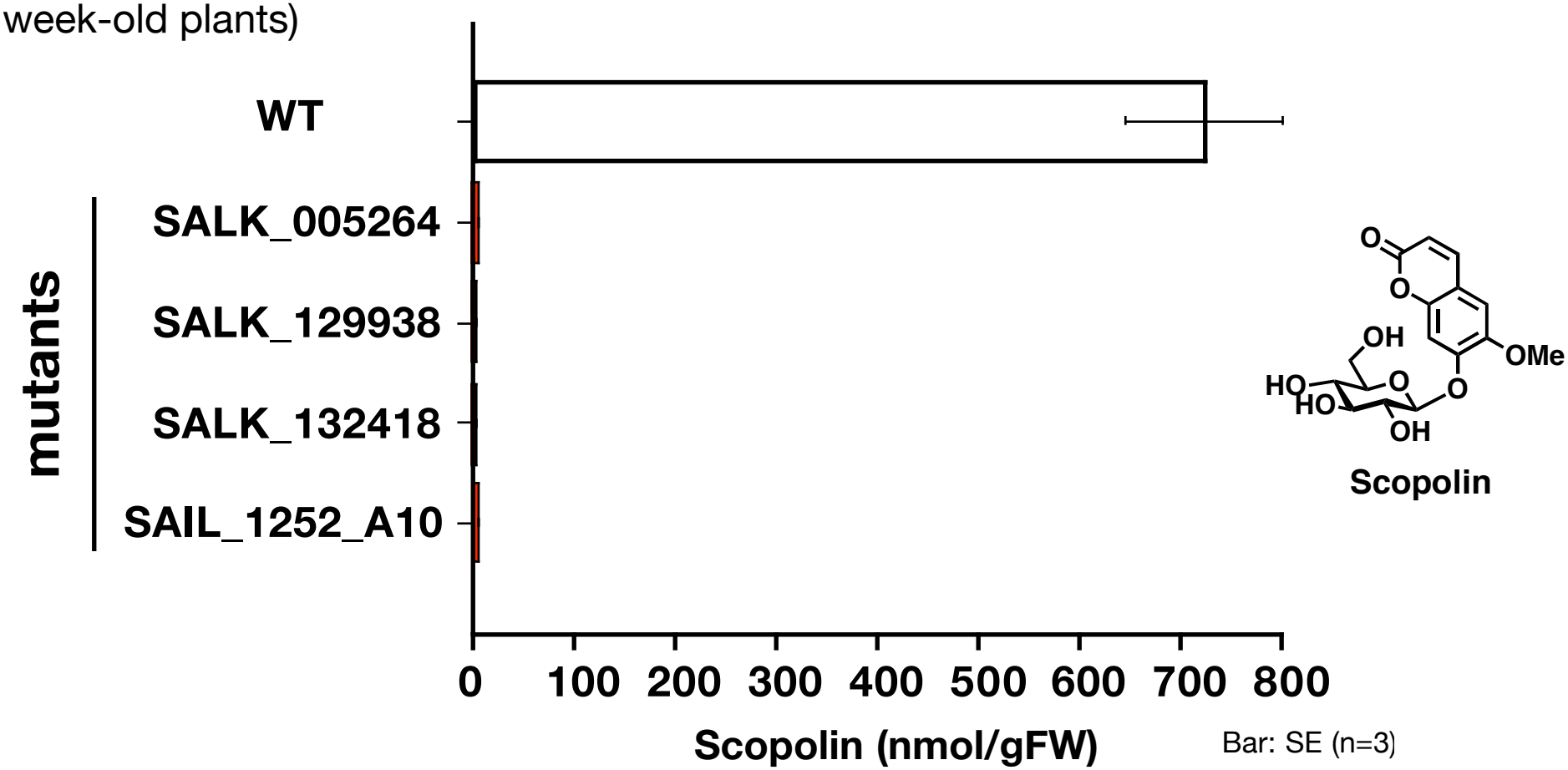
RT-PCR analysis



Plant Journal, **55**, 989-999, 2008

Endogenous level of scopolin in the At3g13610 mutants

(4 week-old plants)



Plant Journal, **55**, 989-999, 2008

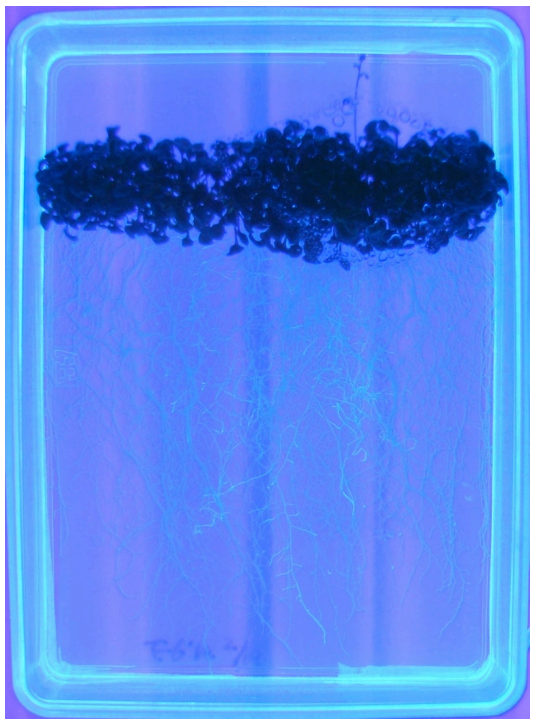
Fluorescence in roots

WT

SAIL_1252_A10

White light

UV



Plant Journal, **55**, 989-999, 2008

Activity of the recombinant F6'H

E. coli BL21 carrying F6'H/pET28a

← 0.5 mM IPTG induction

Homogenize

← Ni-Sepharose column

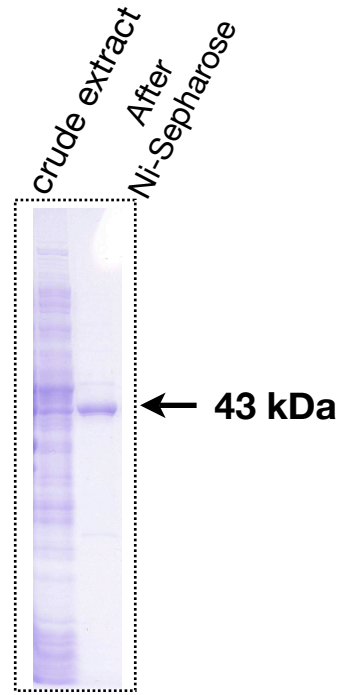
His-tagged protein

Enzyme assay

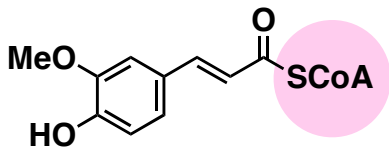
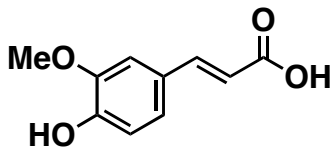
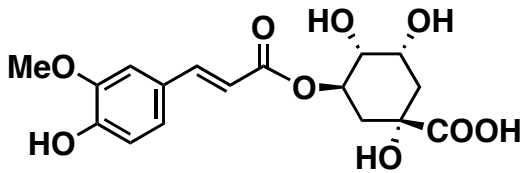
100 mM BisTris-HCl pH 6.5
5 mM $FeSO_4$
50 mM Sodium ascorbate
50 mM 2-Oxoglutaric acid
6 - 192 mM Substrate
1.2 µg protein total 100 µl

- 30°C, 1~5 min.
- ← 30 µl 3N NaOH
- r.t., 30 min
- ← 30 µl 3N AcOH

Reversed-phase HPLC



SDS-PAGE

Substrate		K_m (µM)	k_{cat} (sec^{-1})
Feruloyl CoA		**36.0 ± 4.27**	**11.0 ± 0.45**
Ferulic acid		**N.D.***	
Feruloylquinic acid		**N.D.**	

* N.D. : Product not detected.

Plant Journal, **55**, 989-999, 2008

二次代謝の利用に向けた今後の課題

二次代謝産物の生物学的役割

生合成欠損株（過剰発現体）の
病害抵抗性、ストレス耐性の変化

生理活性発現メカニズムの解明
なぜ抗菌性があるの?

謝辞

京都大学
化学研究所
坂田完三 博士
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（現大阪府大）
玉井道子 氏
山口晃 氏
川村直裕 氏
山本亮太郎 氏
松本征太郎 氏

農学部
石原享 博士

生存研
梅澤俊明 博士
中坪朋文 博士

信州大学繊維学部
田口悟朗 博士

茨城県農業総合センター
渡邊健 博士

小泉商店
小泉邦夫 氏

ご静聴、ありがとうございました.