ABSTRACT : 209

EXPRESSION HETEROGENEITY OF KEY GENES OF THE ISOFLAVONE BIOSYNTHETIC PATHWAY IN MATURATING SOYBEAN SEED

BERGER M, ARTIGOT M P, DAYDÉ J

Université de Toulouse, Ecole d'Ingénieurs Purpan, Toxalim UMR 1331 INRA/INP/UPS - 75, voie du TOEC - BP 57611 – 31076 Toulouse cedex 3 – France

E-mail: monique.berger@purpan.fr

Soybean seed stores large amounts of three isoflavones (genistein, daidzein and glycitein). They are not uniformly deposited in the seed tissues. The total content in the hypocotyl (embryo axis) is 4 to 10 times higher than that of cotyledons and these seed parts have their own specific composition. The cotyledons contain only genistein and daidzein (more than 50% genistein), whereas the hypocotyls contain the three isoflavones, but daidzein and glycitein account for 90% of the total isoflavone content. The isoflavones do not accumulate simultaneously in the seed parts. The accumulation begins in cotyledons once achieved in the hypocotyls. Previous studies have revealed two main phenotypes for the glycitein and daidzein percentages in the hypocotyl (35% or 55% glycitein). The segregation for this trait suggests control by a single or perhaps two genes. Understanding the determinism of this seed heterogeneity should lead to better control of the isoflavone content and composition in seed parts which can be distinct raw materials in the food industry.

Two studies are presented here: 1) the expression of key genes of the isoflavone biosynthesis pathway followed at critical stages of accumulation in hypocotyls and cotyledons of developing seeds of two contrasted cultivars for their total isoflavone content in cotyledons and their isoflavones composition in hypocotyls and cotyledons and 2) the expression of putative candidate genes coding for a flavonoid 6-hydroxylase which catalyzes the first step of glycitein biosynthesis studied in 4 genotypes presenting a comparable isoflavone total content in hypocotyls but 0, 35, 55 or 80% glycitein.

The isoflavone synthase genes were less expressed in hypocotyls than in cotyledons. The expression of the chalcone synthases *CHS7* and *CHS8* was related to the isoflavone accumulation in cotyledons, but not with the total content at maturity. *CHS9* was the most expressed CHS isoform, and only correlated with the hypocotyl isoflavone accumulation kinetics. The candidate genes *F6H1* and *F6H2* were not expressed in the developing seed. *F6H3* was expressed only in hypocotyls but not in the null mutant for glycitein.

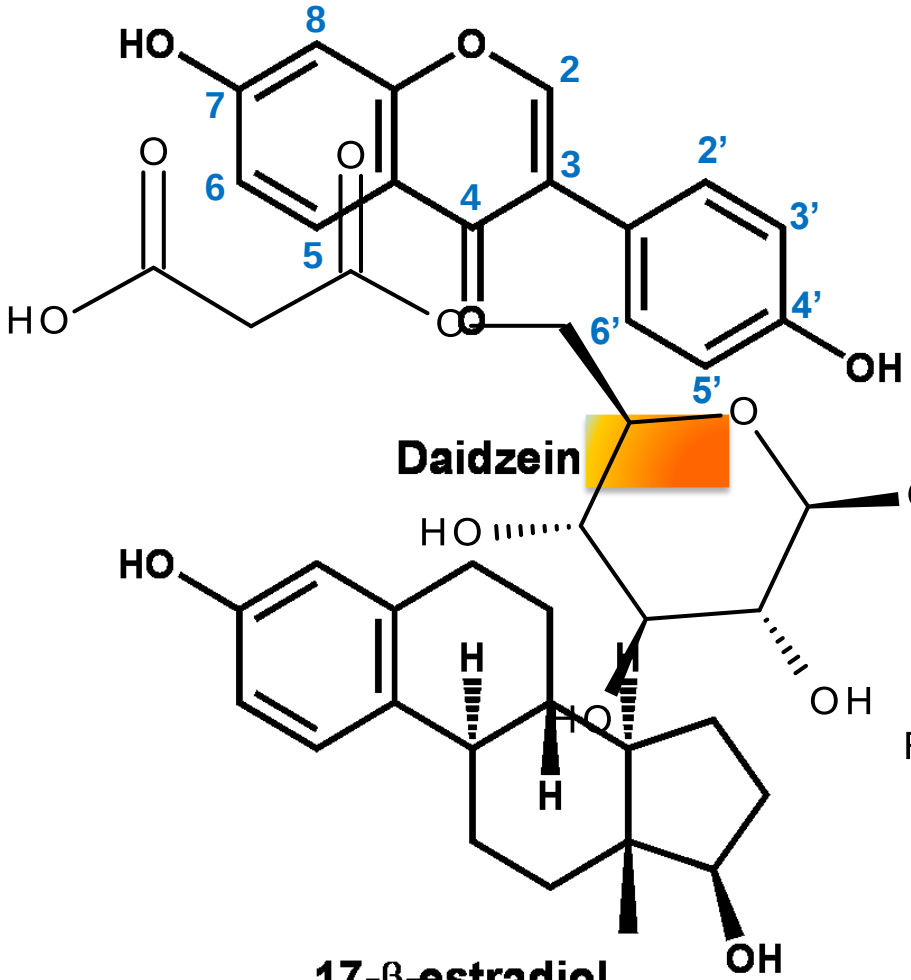
These results indicate that the isoflavone biosynthesis pathway is under distinct control in cotyledons and hypocotyls during seed maturation. The *F6H3* gene is a good potential candidate for the flavonoid 6-hydroxylase function in the soybean seed.

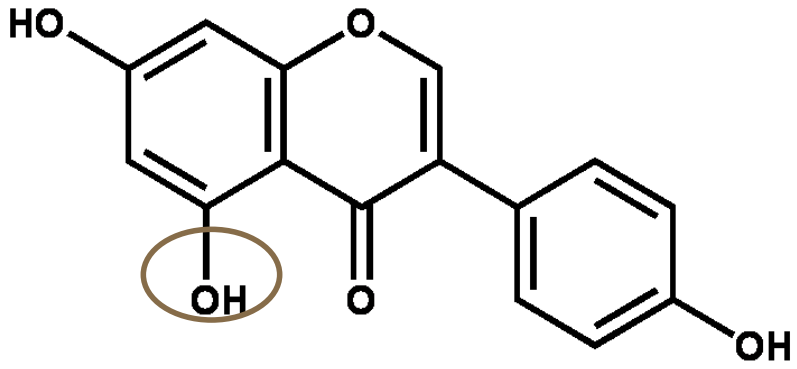
EXPRESSION HETEROGENEITY OF KEY GENES OF THE ISOFLAVONE BIOSYNTHETIC PATHWAY IN MATURATING SOYBEAN SEED

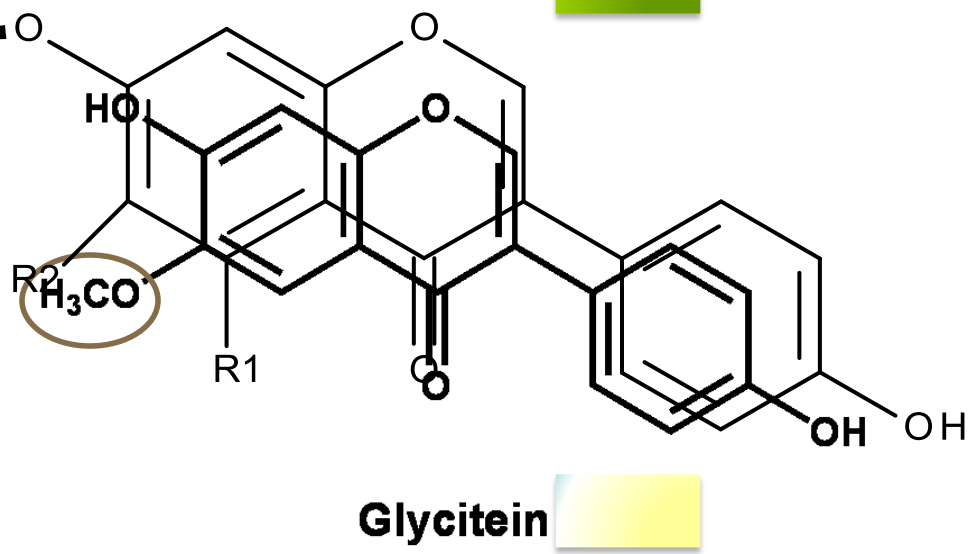
Monique BERGER, Marie-Pierre ARTIGOT and Jean DAYDÉ

Université de Toulouse, INP - E.I Purpan, FRANCE
Laboratoire Agrophysiologie – Toxalim UMR 1331 INRA/INP/UPS

Isoflavones







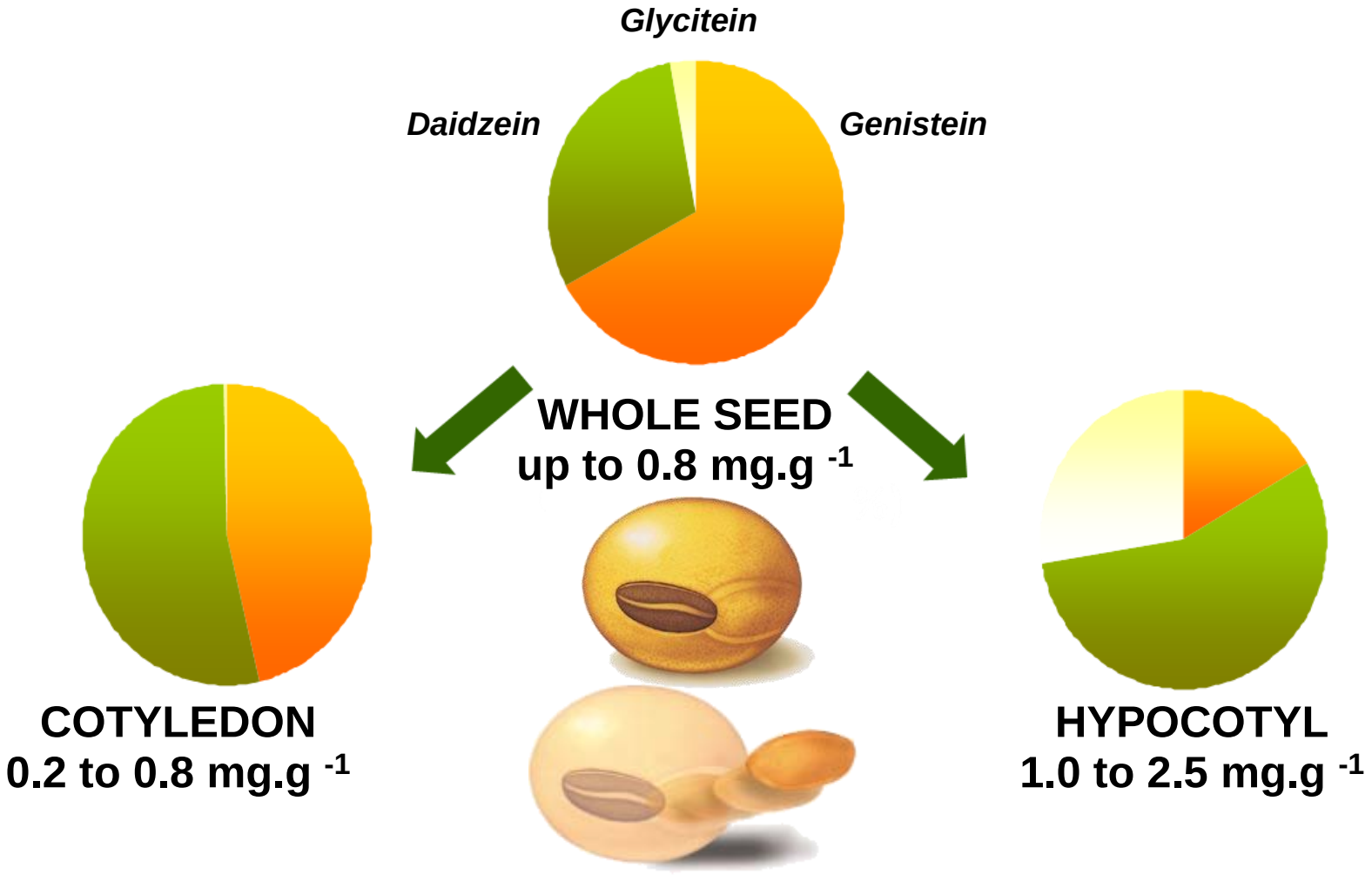
Isoflavones and human nutrition

- **Potential role in human health**
  - Protective effects against hormone dependant cancers, cardiovascular disease, osteoporosis, menopausal symptoms
  - Phytoestrogenic activities =>potential endocrine disruptors ?

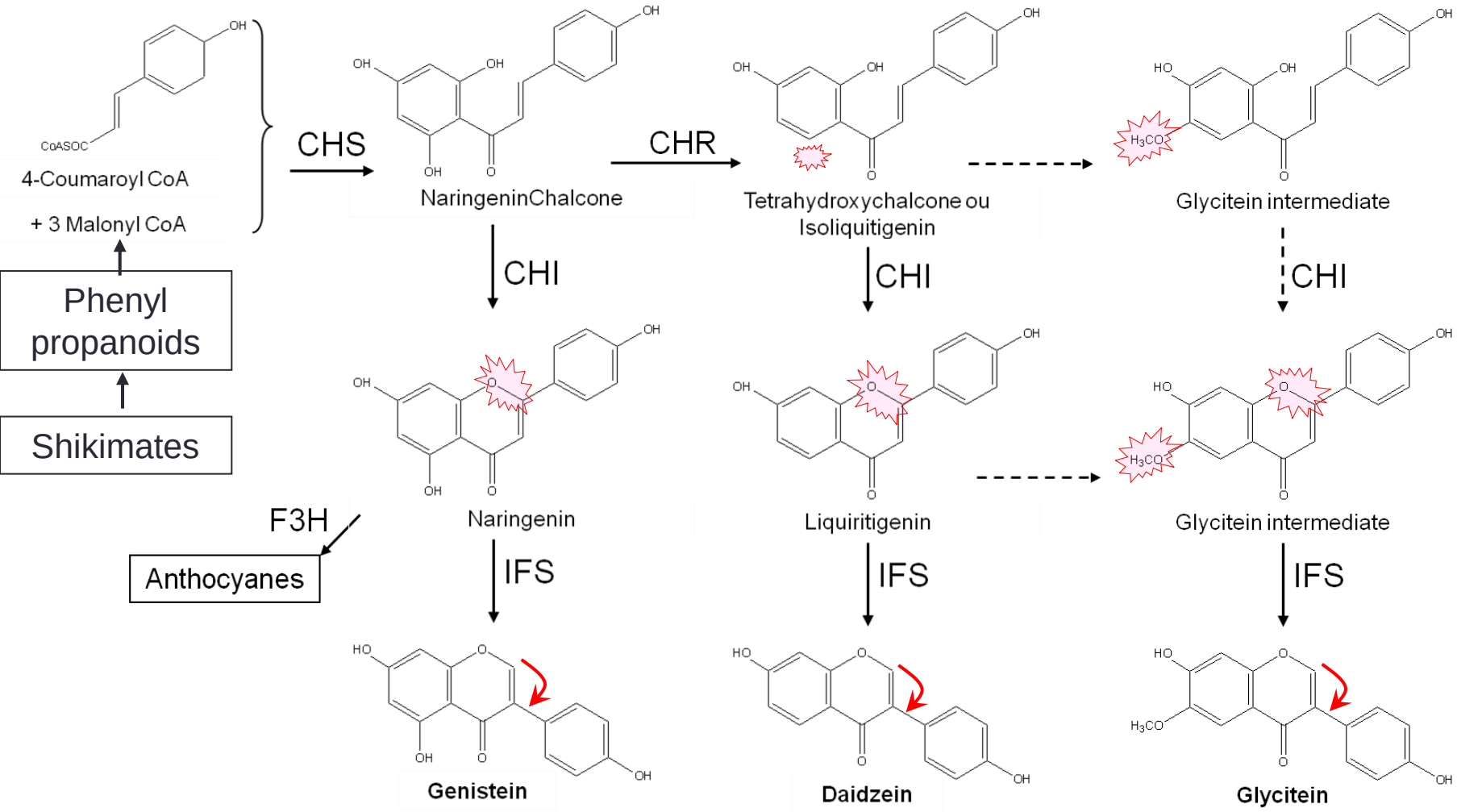
(Manach et al.2007;Eisenbrand 2007,Ferrari 2009;Li et al.2010; Prasad et al.2010)

- **Two kinds of industrial demand**
  - Low isoflavone content for infants formulas
  - Mid or high isoflavone content for specific health markets (functional food for woman, elderly, vegetarians…)

Isoflavones in the seed



Isoflavones biosynthesis



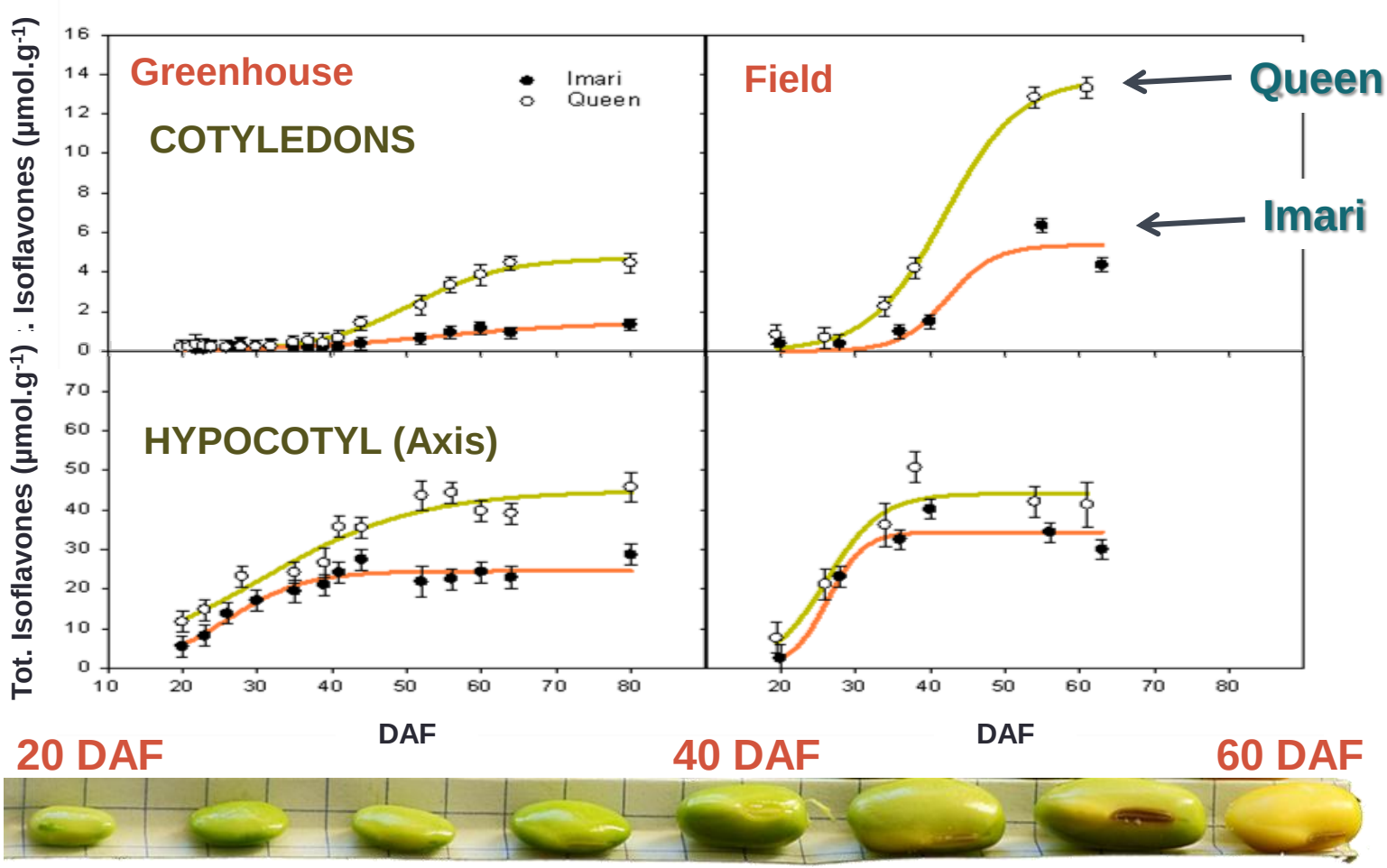
Objectives

- Study the isoflavone biosynthesis genes in the two seed fractions to explain
  - The differences in the time-curve of isoflavone accumulation between hypocotyl and cotyledons
  - The 10-fold gap between their isoflavone contents
- Study flavonoid 6-hydroxylase genes, candidates for the first step of glycitein biosynthesis

Plant material (1)

I. Two contrasted cultivars : Imari and Queen

Very different IF contents, whatever environmental conditions *(Rasolohery et al.2007)*



Methods (1)

I - *IFS1* and *IFS2* gene isolation and characterization

- PCR amplification + Cloning (TA TOPO vector) + sequencing (3 replicates)

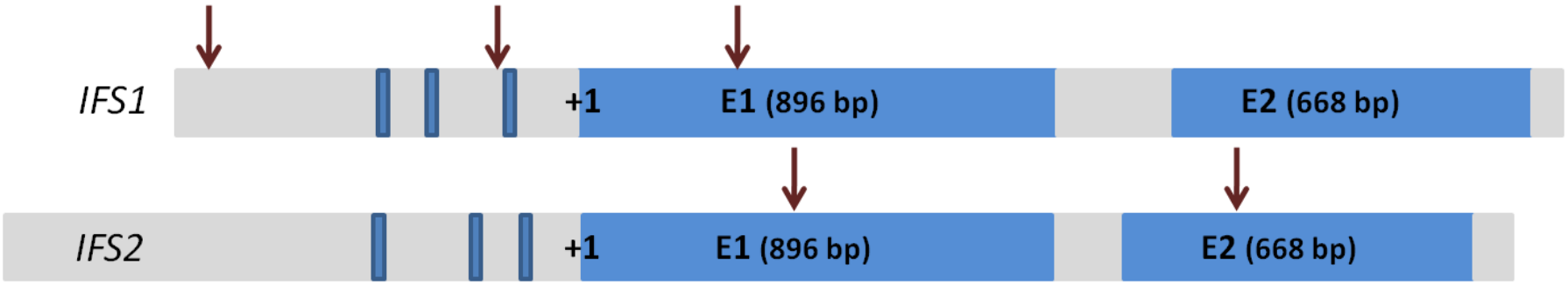
II - Expression study of isoflavone pathway key genes

from 25 DAF to maturity (≈ 70 DAF)

- Plants grown in **greenhouse**
- **2 biological samples per stage** (pool of 10 seed fractions from 2 or 3 plants)
- **Isoflavone analysis** on 6 stages (25, 32, 40, 50, 60, 70 DAF)
- **qPCR for 3 stages** (25, 40 and 60 DAF) **and 16 genes** + 2 housekeeping genes (β-tubulin and actin)

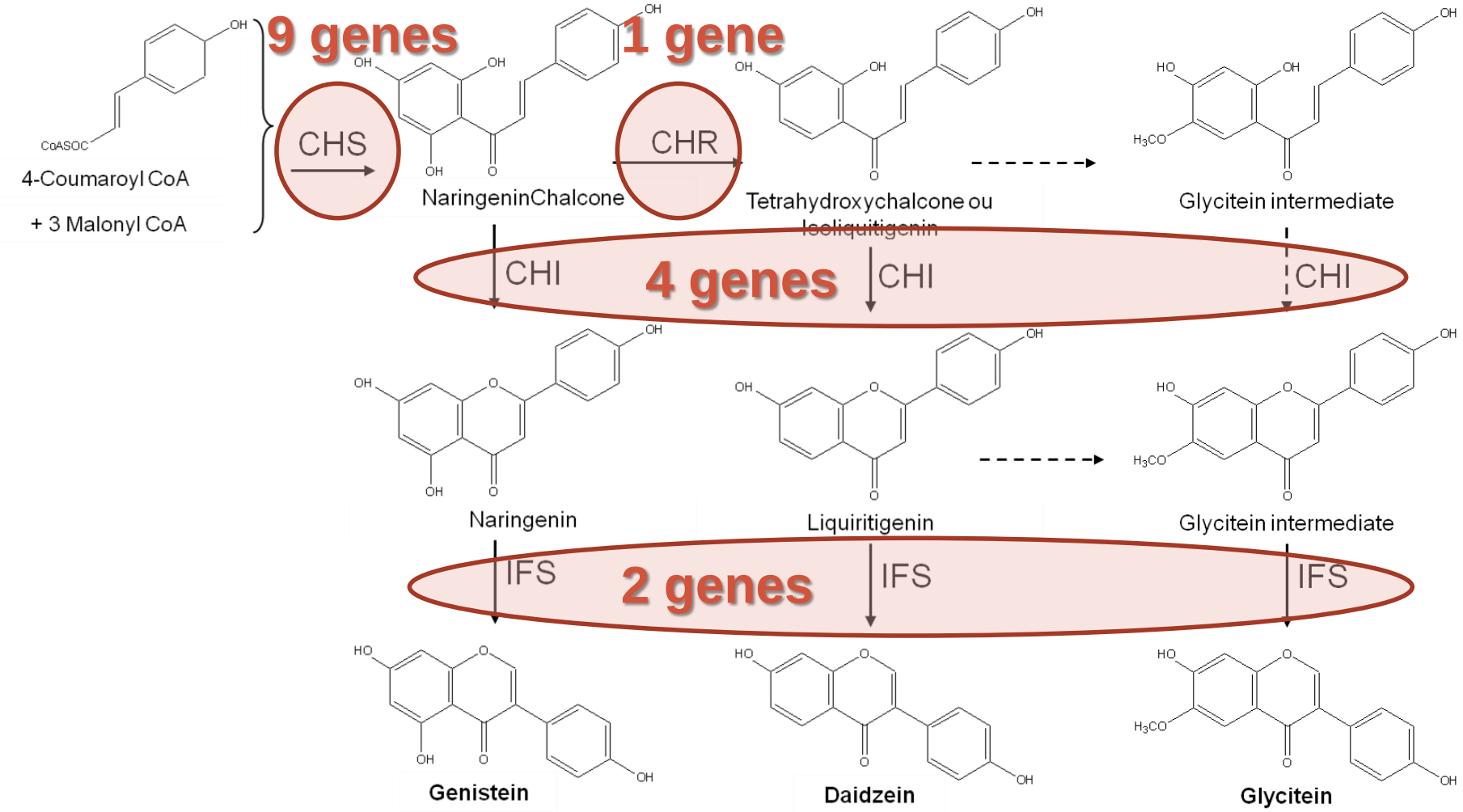
I - IFS1 and IFS2 sequence analysis and comparison between Imari and Queen

- 5 SNPs have been correlated with isoflavone content
  (Cheng et al. 2008)

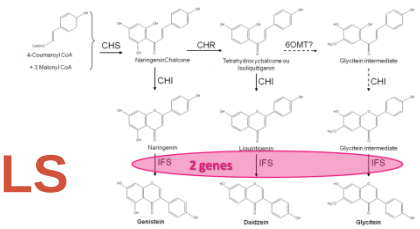


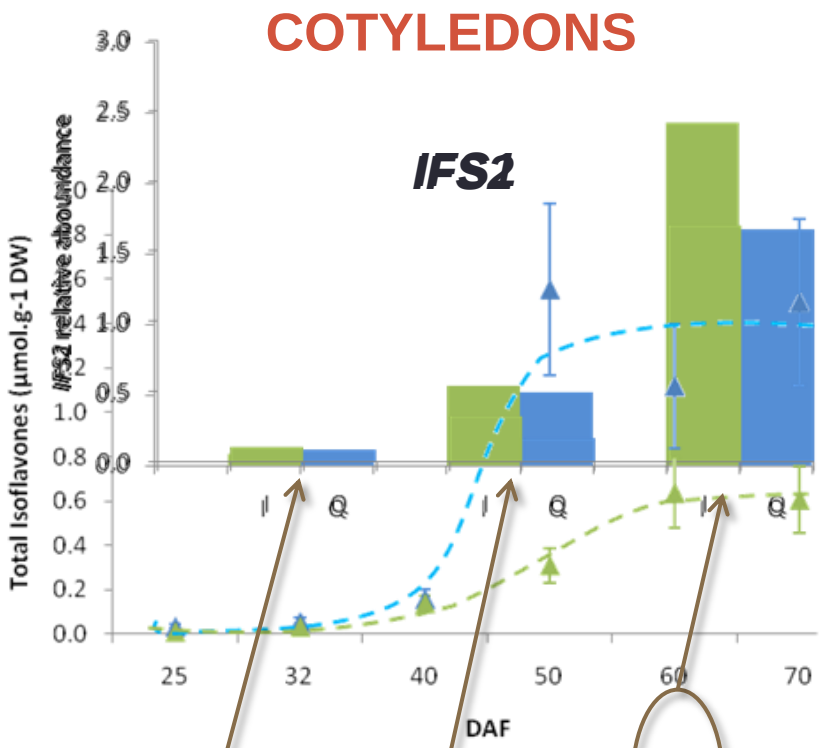
Despite their contrasted phenotypes, cv. Imari and Queen have exactly the same *IFS1* and *IFS2* sequences

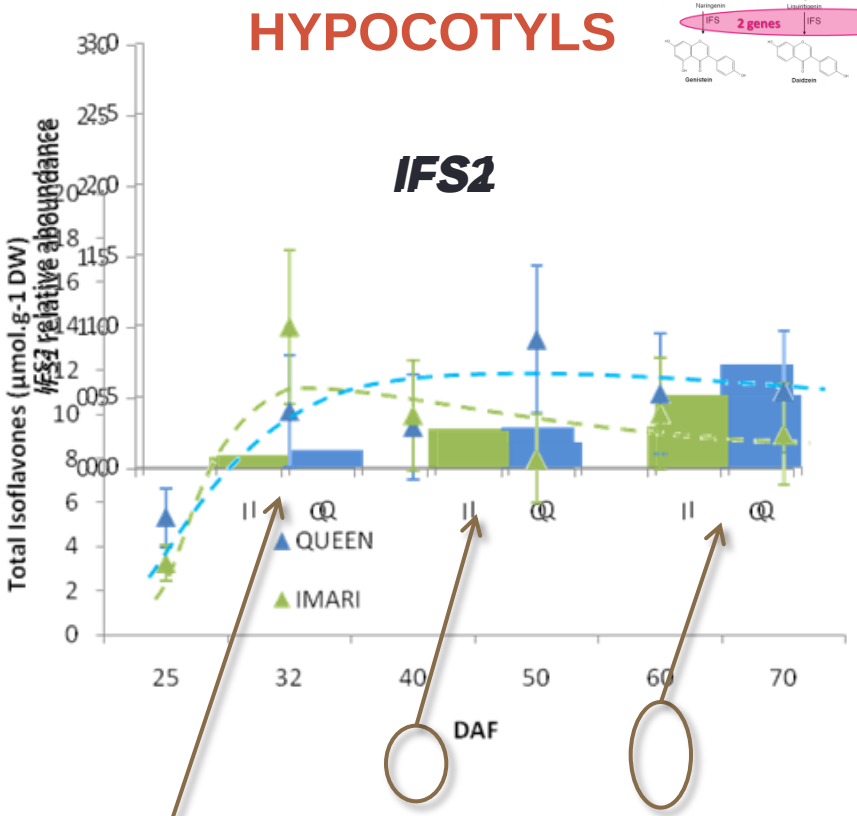
II – Isoflavones pathway genes expression



II.1 – Isoflavone synthases





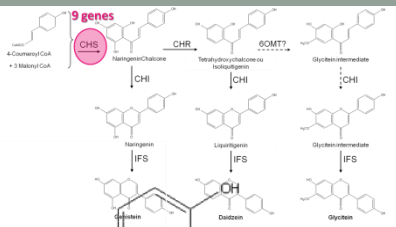


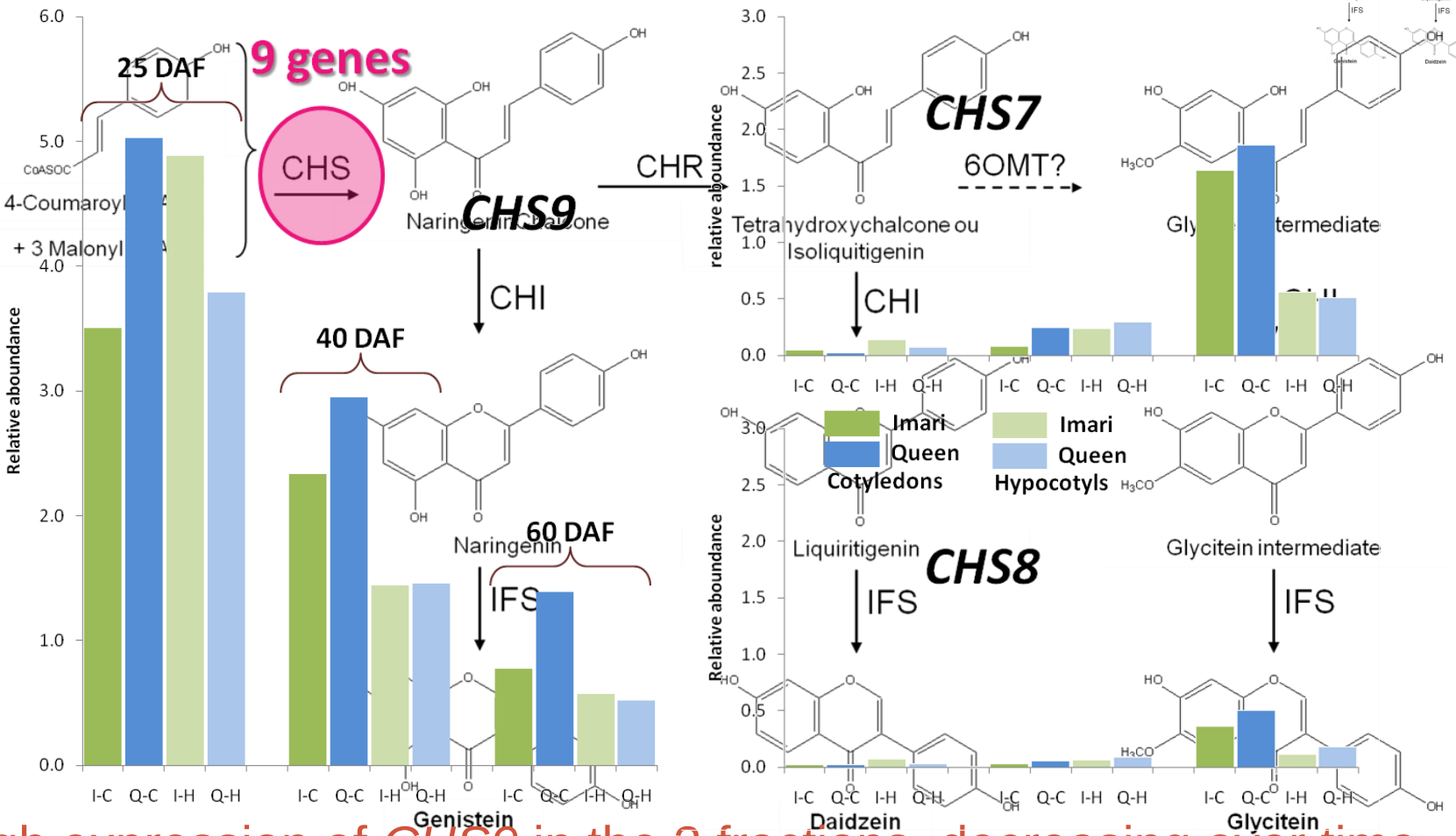
- Isoflavone content
  - In cotyledons, cv. Queen has twice as much IF as cv. Imari (maturity)
  - 10 times higher in hypocotyl than in cotyledons

IFS2 -> IF content among different environmental conditions *(Gutierrez-Gonzalez et al. 2010)*

***IFS1* and *IFS2* are not responsible for cv. and fraction differences in IF content**

II.2 – Chalcone synthases





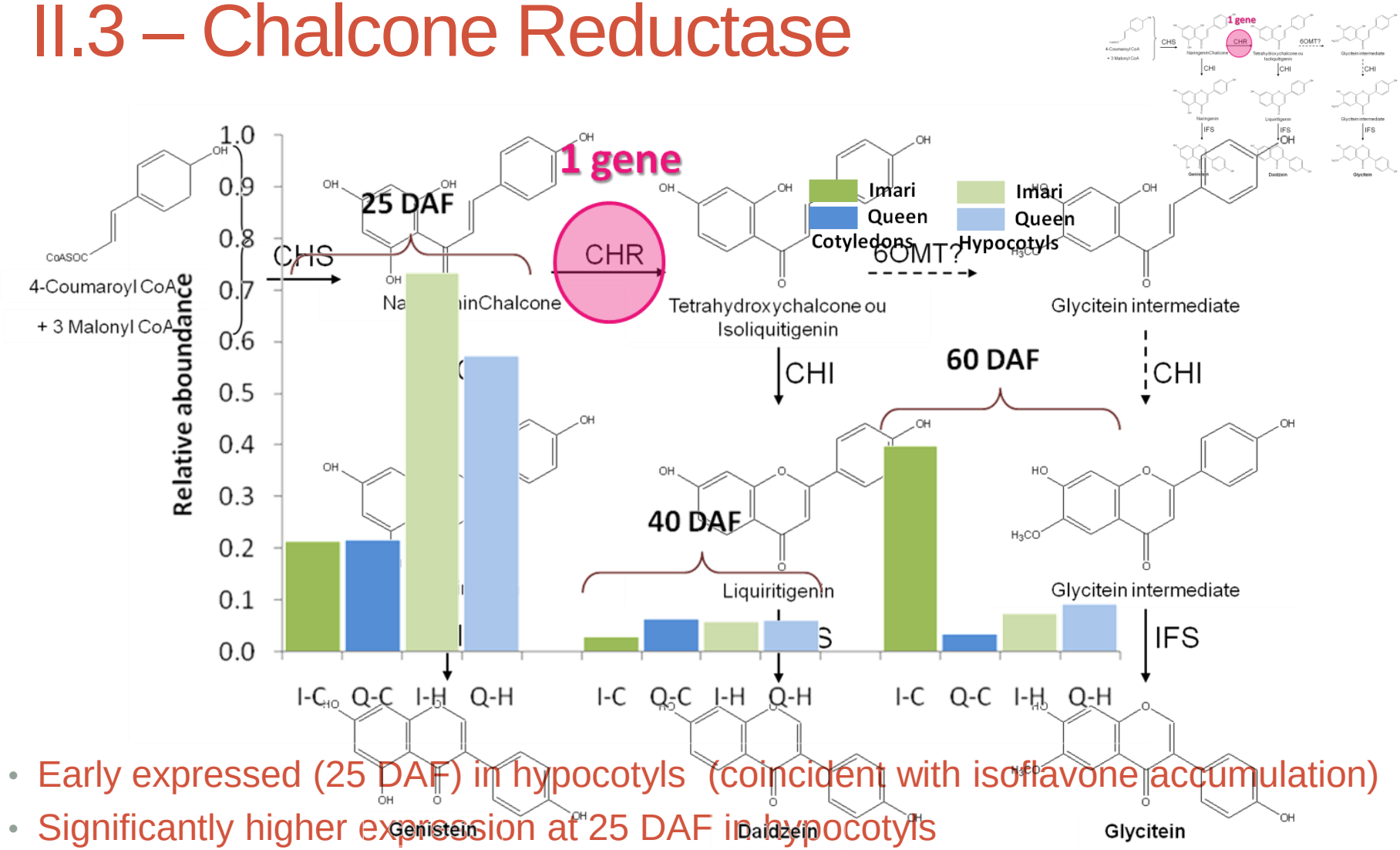
High expression of *CHS9* in the 2 fractions, decreasing over time

In cotyledons, the expression of *CHS9* is significantly higher in Queen than in Imari

CHS7 and *CHS8* are seed specific and correlated to isoflavone content *(Gutierrez- Gonzalez et al. 2010)*

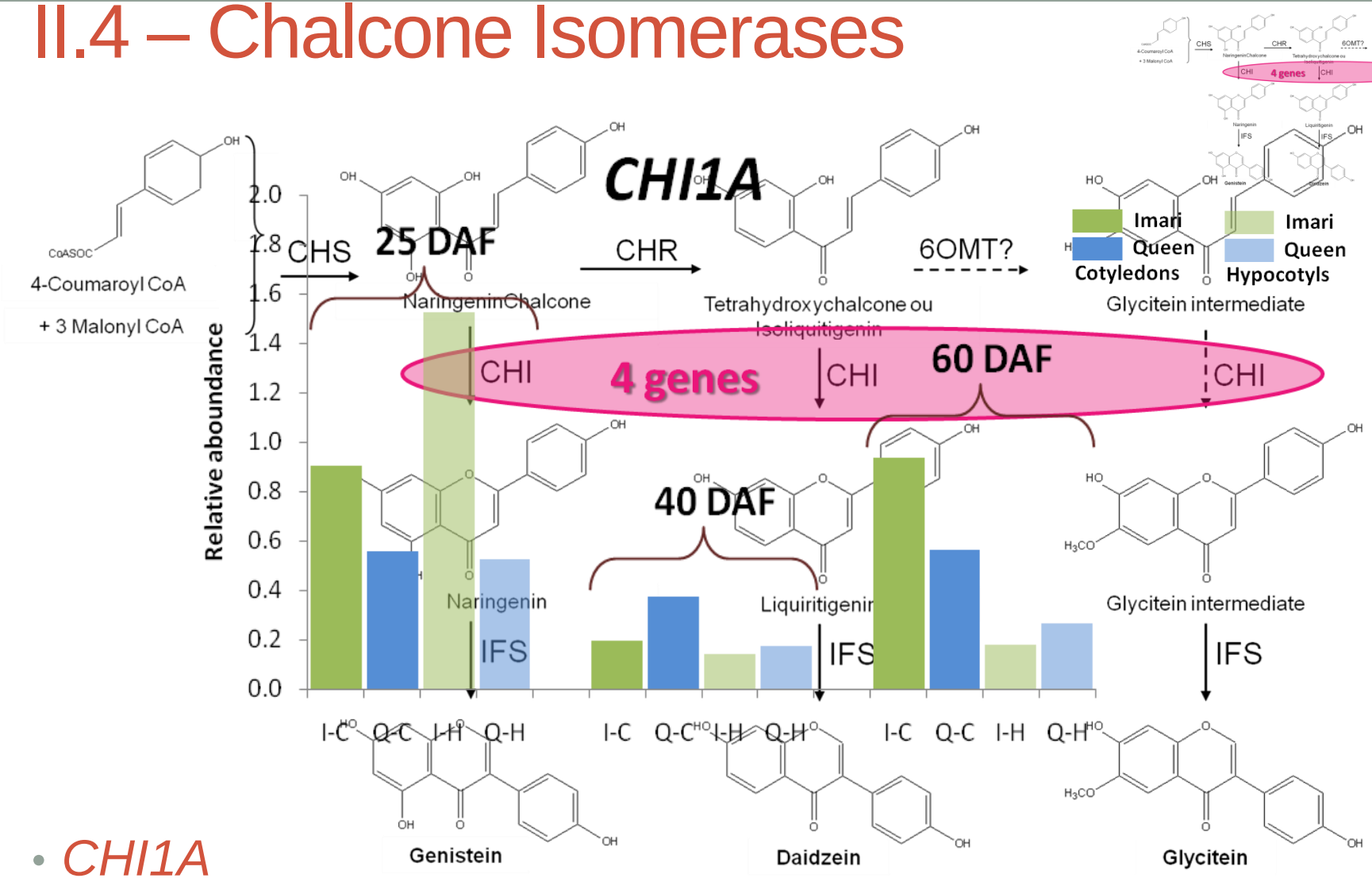
-> their expression increased over time, with the highest level in cotyledons at 60 DAF

II.3 – Chalcone Reductase



- Early expressed (25 DAF) in hypocotyls (coincident with isoflavone accumulation)
- Significantly higher expression at 25 DAF in hypocotyls
- And remarkably, daidzein and glycitein (which depend on CHR activity) are the main hypocotyl isoflavones

II.4 – Chalcone Isomerases

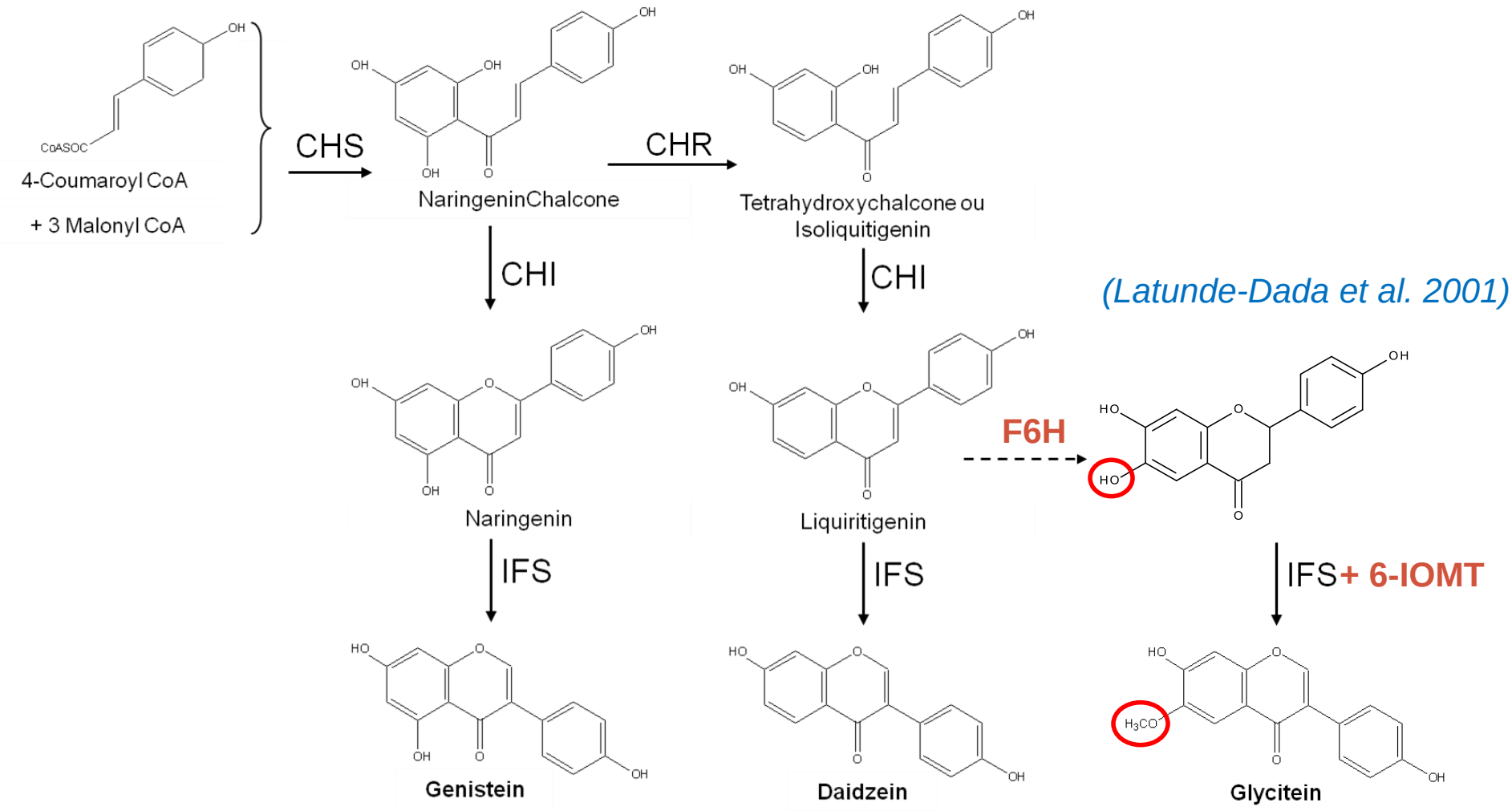


- *CHI1A*
  - significantly higher expression at 25 DAF (cv. Imari - axis)
  - higher expression in cotyledons than in hypocotyl at 60 DAF

Summary and conclusion of this part

- Isoflavone synthase gene expressions were not correlated with total IF contents
- For most of the genes considered, a higher expression was found in cotyledons *versus* hypocotyls. This finding strongly contrasts with data showing 4 to 10 times higher isoflavone accumulation in hypocotyls.
- *CHS9* and *CHR* could be the key genes of isoflavone accumulation in hypocotyls
- *CHS7*, *8* and *9* were the only CHS genes for which expression correlates with cultivar differences in isoflavone contents
- *CHI1A* displayed cultivar differences which suggest further investigations for its chalcone specificities and/or limiting activity in the pathway

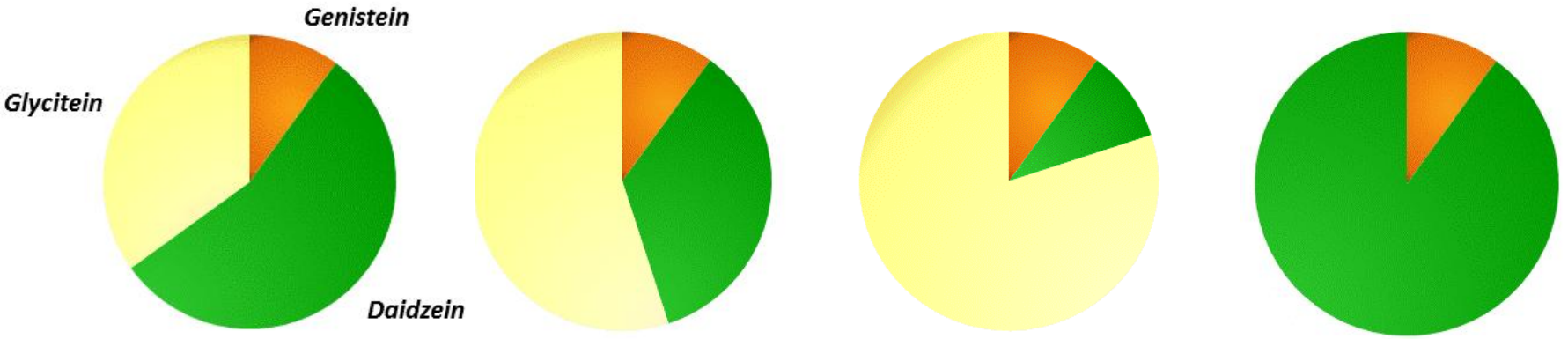
The Glycitein pathway



(Latunde-Dada et al. 2001)

Plant material (2)

II. Four contrasted cultivars with different glycitein content in hypocotyl



Imari	Queen	NS Kasha	PI567580A
Glycitein ≈ 35%	Glycitein ≈ 55%	Glycitein ≈ 80%	Glycitein Null

Methods (2)

- **I - In silico**
  - Exploration of genomic sequences of *Glycine max*, *Phaseolus vulgaris* and *Medicago troncatula* for gene sequences similar to the unique F6H sequence published (Genbank Y10490)
  - Classification and rooted tree construction using ClustalW 2.0.12 multiple alignment and Phylip 3.67 drawgram

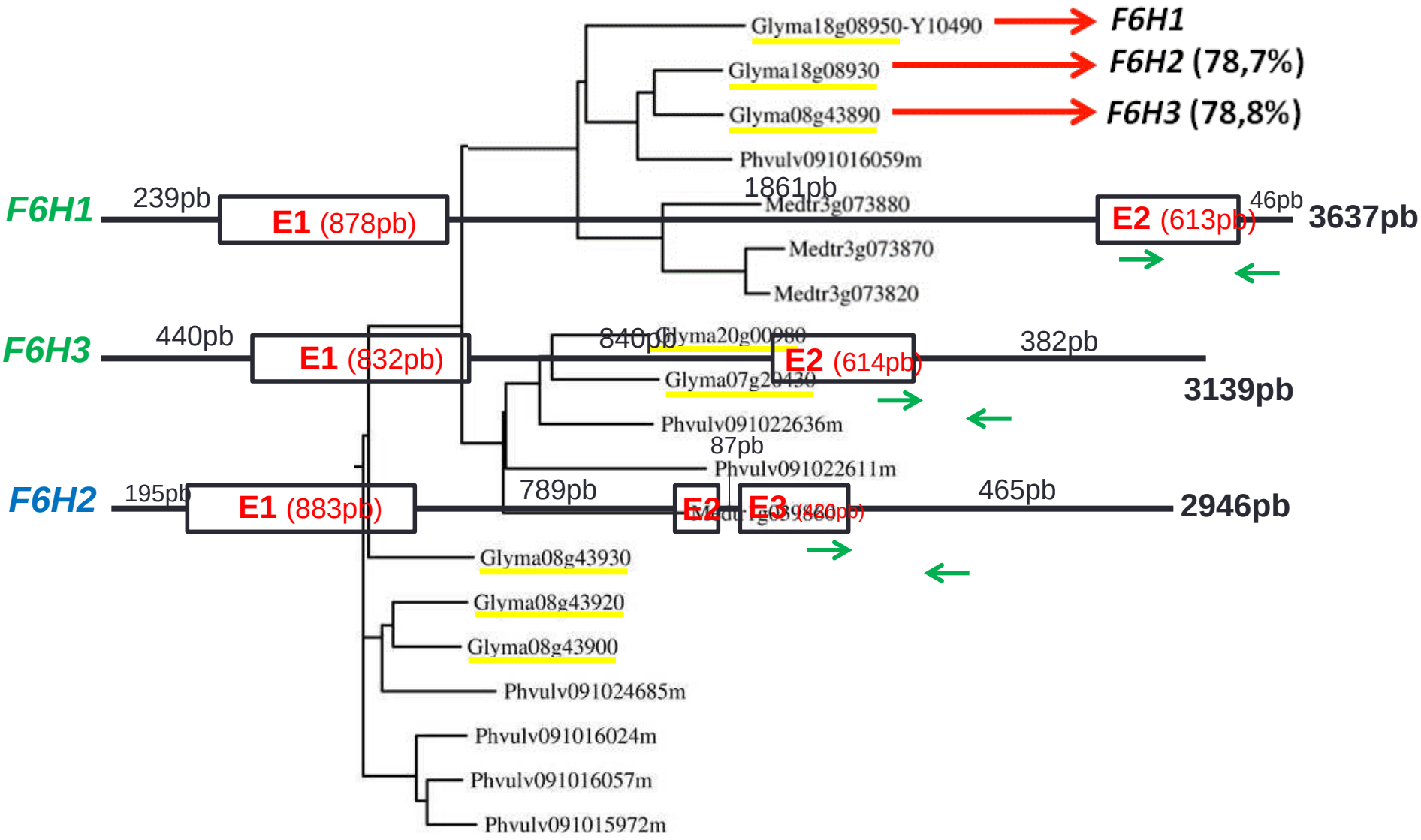
II – Sequencing

- The selected genes have been cloned and sequenced

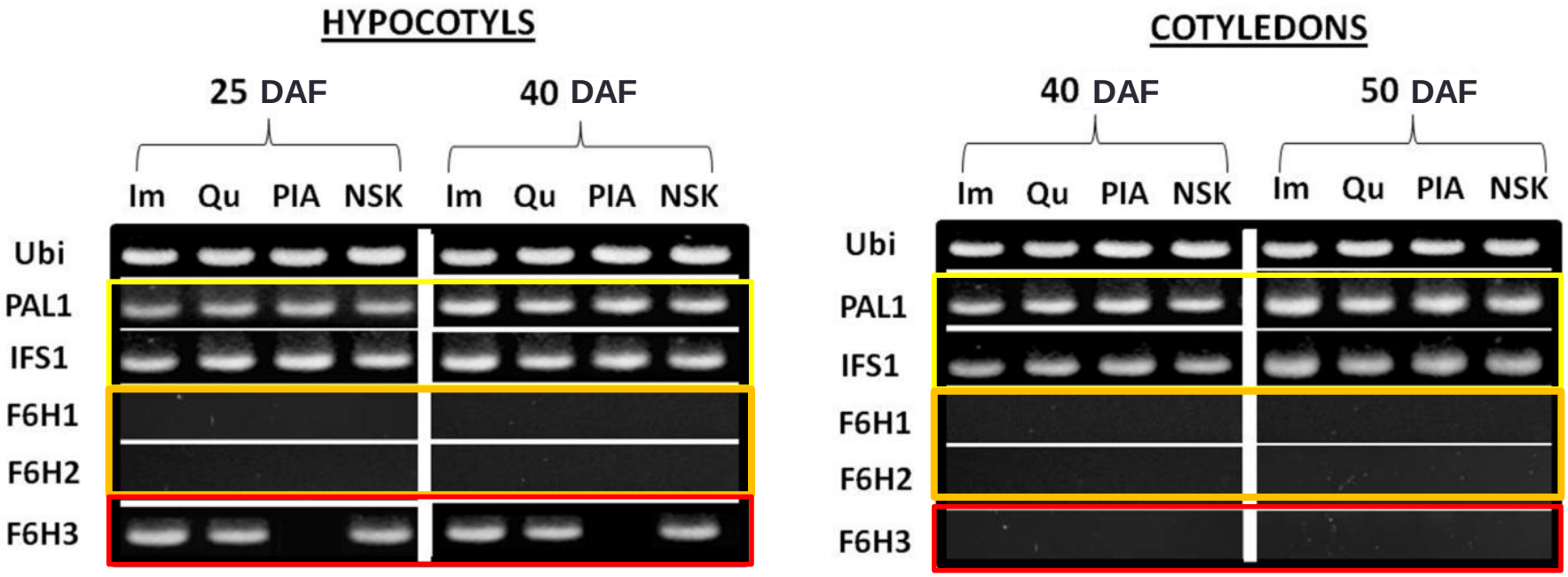
III – RNA extractions and RT-PCR

- In developing seeds of the 4 genotypes, at 25 and 40 DAF (hypocotyls) or 40 and 50 DAF (Cotyledons)

Candidate genes



Expression in developing seed



- The 2 controls ***(PAL1, IFS1)*** are correctly expressed in the seed of the 4 cultivars

- ***F6H1*** and ***F6H2*** were not detected in the seed

-***F6H3*** is expressed only in the hypocotyl, but not in the glycitein null mutant

Summary and conclusion of this part

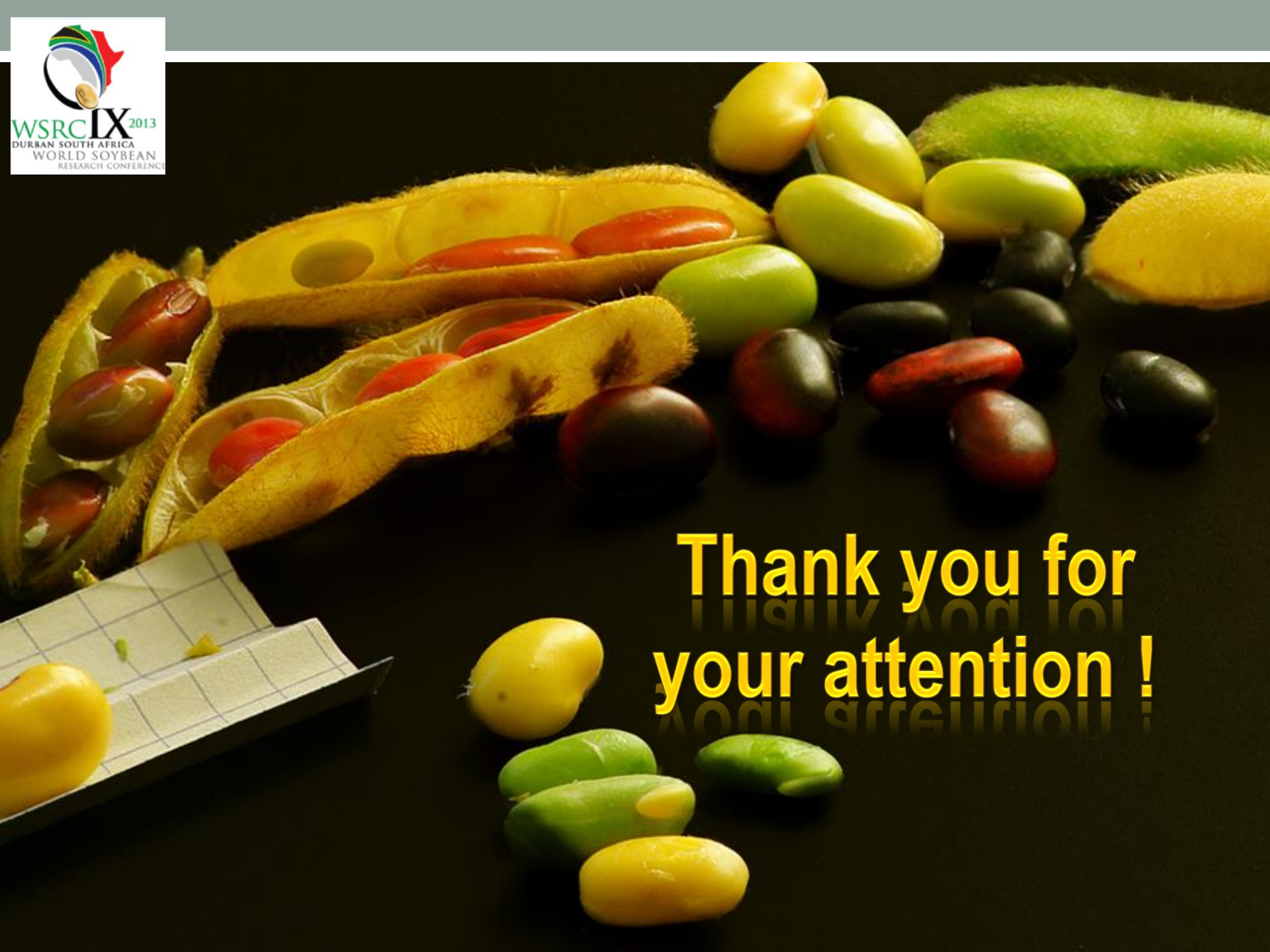
- F6H1 have been identified in RNAs of elicited soybean leaves and is not expressed when the seed accumulates isoflavones
- F6H3 is expressed in the leaves and in the hypocotyl, and not in a glycitein null mutant.
- F6H3 may be a good candidate, but need further characterization for its substrate specificity and its interaction with the membrane and IFS

General Conclusion

- The implication of the isoflavone synthases in the control of total isoflavone content was not observed here. In fact, it had been shown with wild germplasms.
- The glycitein pathway may be initiated by a F6H function, but its possible interaction IFS, another P450 needs further investigations
- The specific control and gene expressions in cotyledons and hypocotyl may account for a part of the complex epistatic interactions between some QTLs for isoflavone individual and/or total content

Acknowledgments

- Marie Pierre Artigot
- Mathieu Baes
- Jean Daydé
- Vassilia Théodorou
- Alban Jacques
- Christel Couderc
- Clémence Loubet
- Olivier Puel
- Arnaud Polizzi


WSRC IX 2013
DURBAN SOUTH AFRICA
WORLD SOYBEAN
RESEARCH CONFERENCE
Thank you for
your attention !