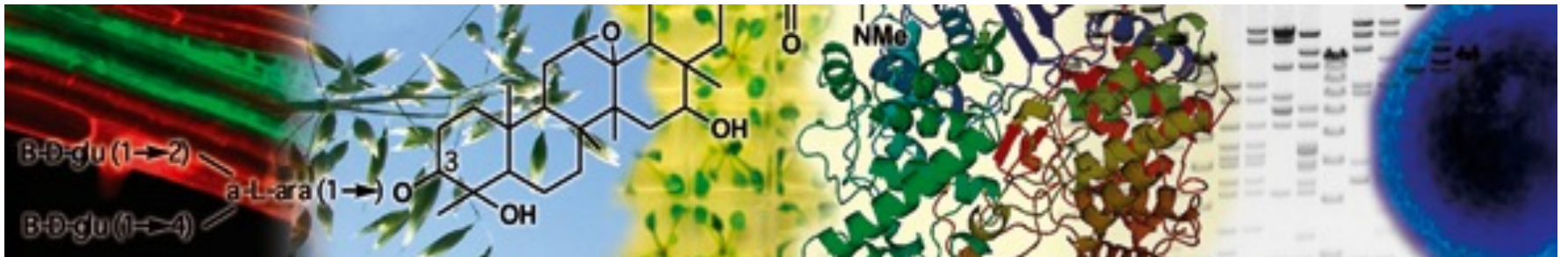


# Making new molecules

Anne Osbourn  
anne.osbourn@jic.ac.uk



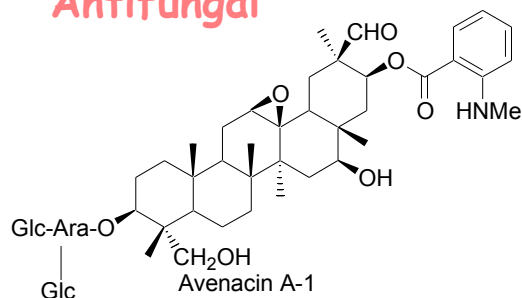
# Understanding and Exploiting Plant and Microbial Metabolism (MET)



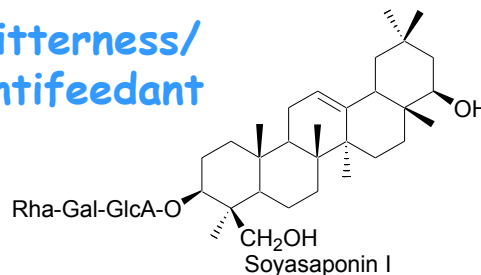
The MET ISP - Critical mass in metabolism  
BBSRC Award No. BB/J004561/1 - £23.8m

# Metabolic diversity - a snapshot

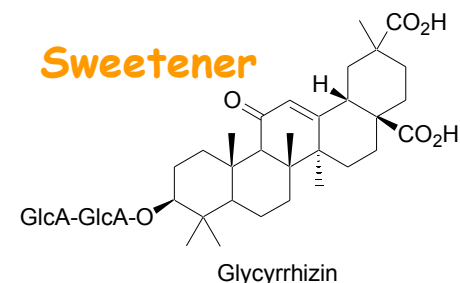
Antifungal



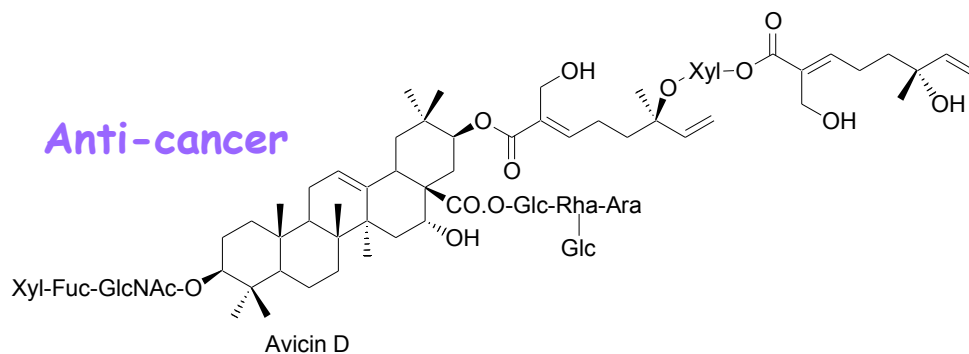
Bitterness/  
antifeedant



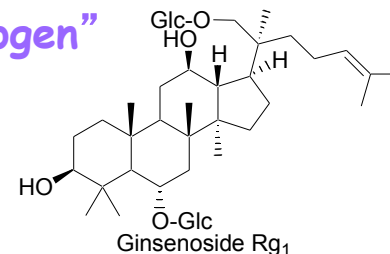
Sweetener



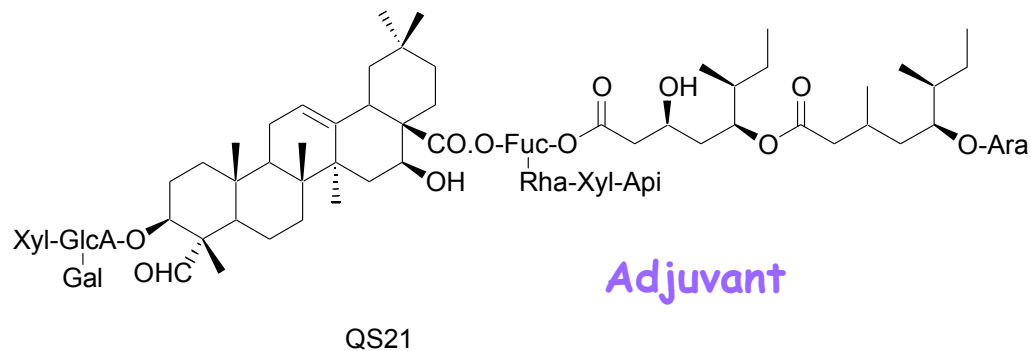
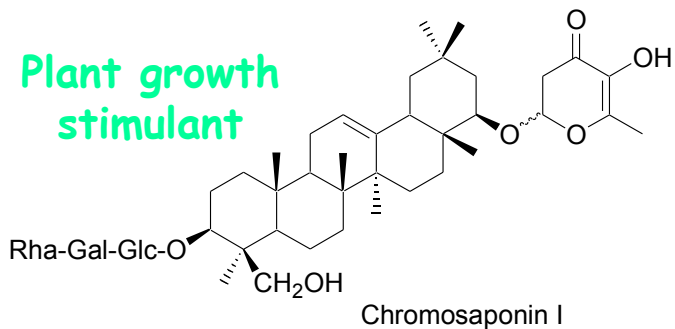
Anti-cancer



“Adaptogen”



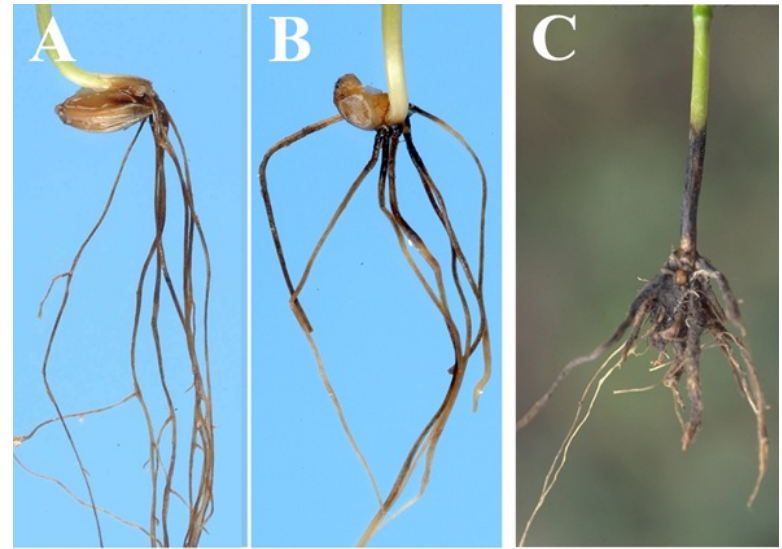
Plant growth  
stimulant



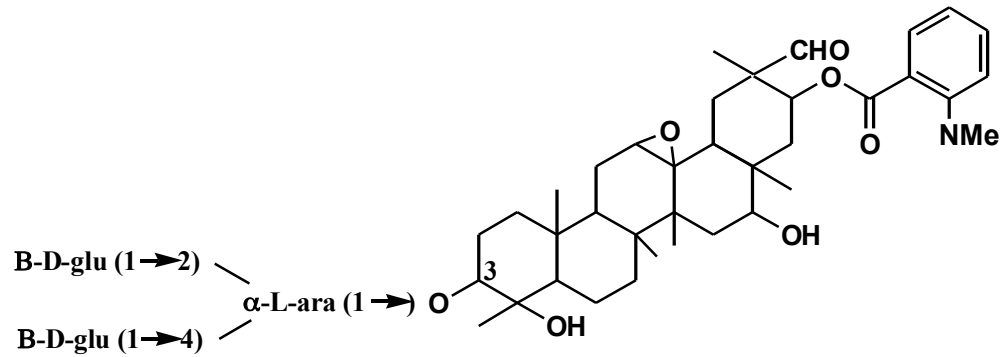
Adjuvant

# Take-all disease of wheat

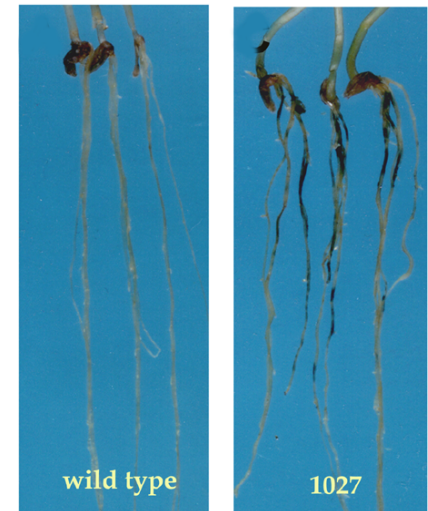
- The most damaging root disease of wheat worldwide
- The most widespread and costly disease problem faced by UK cereal growers
- Conservative estimate of take-all associated yield losses in the UK £85 m - £340 m



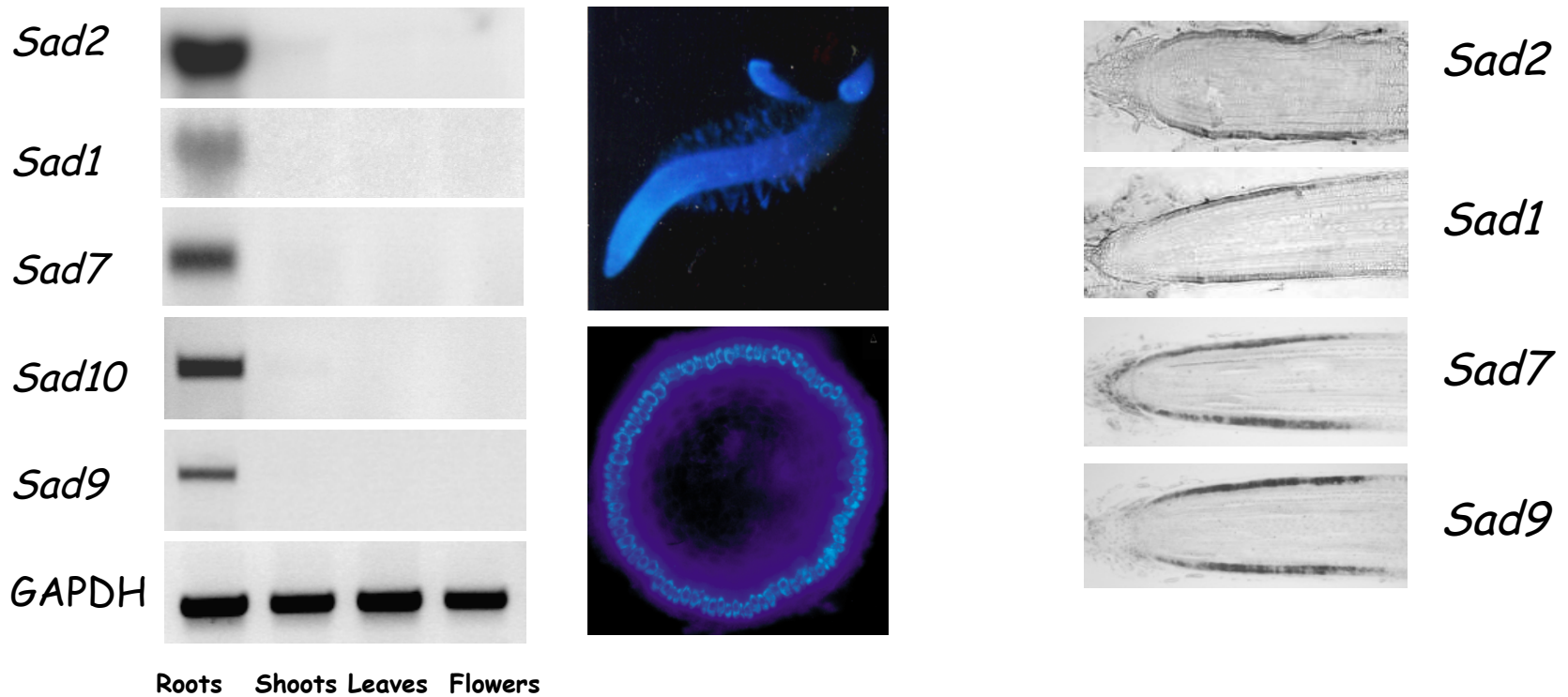
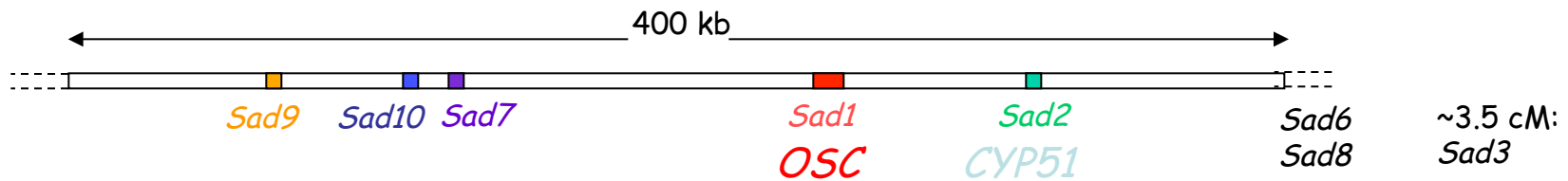
# Avenacins - Antimicrobial defence compounds produced by oat (*Avena* spp.)



Avenacin A-1

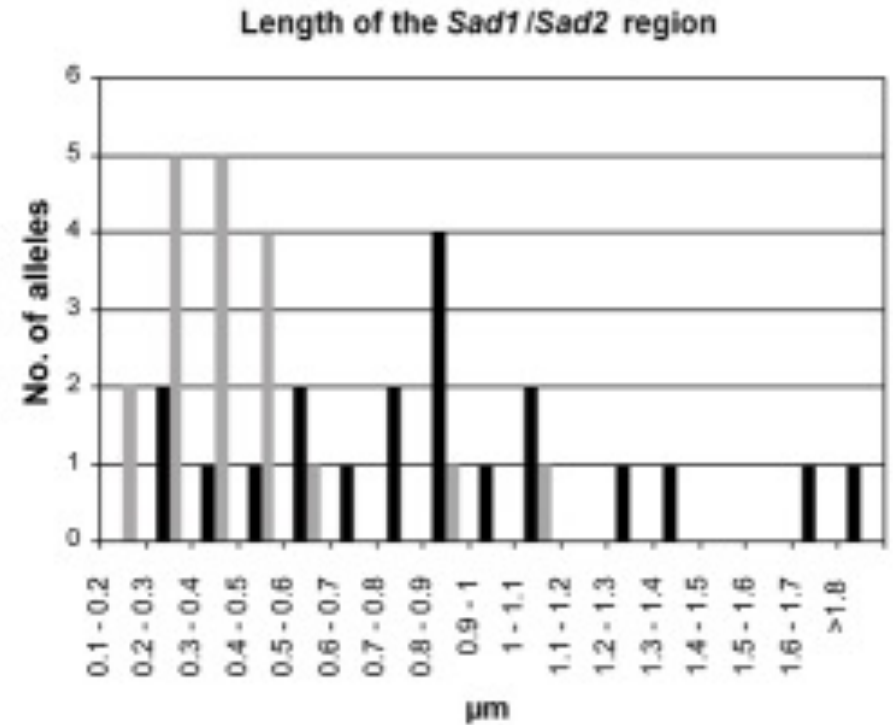
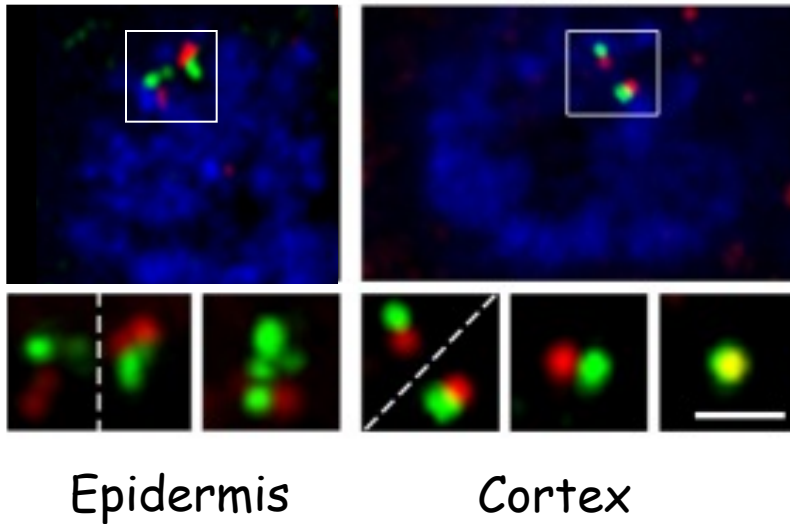
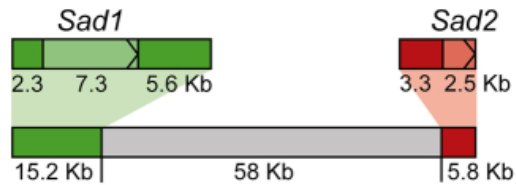


# The genes for avenacin synthesis are clustered



Qi et al (2004) PNAS 101:8233; Qi et al (2006) PNAS 103:18848; Mylona et al (2008) Plant Cell 20:201; Mugford et al (2009) Plant Cell 21:2473; Wegel et al (2009) Plant Cell 21:3926; Qin et al (2010) Phytochemistry 71:1245; Owatworakit et al (2012) JBC, Dec 20<sup>th</sup>; Mugford et al Plant Cell, March 2013.

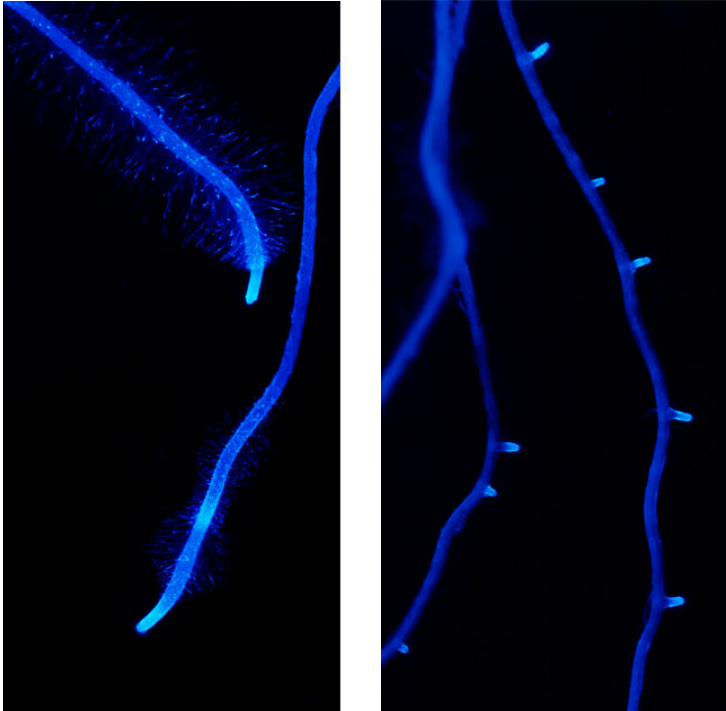
# DNA FISH - Gene expression is associated with chromatin decondensation



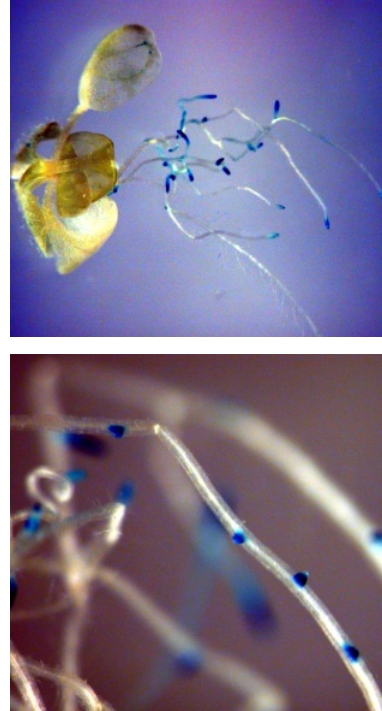
■ Epidermis (n=20)  
 ■ Cortex (n=20)

# The oat promoters are active in other species

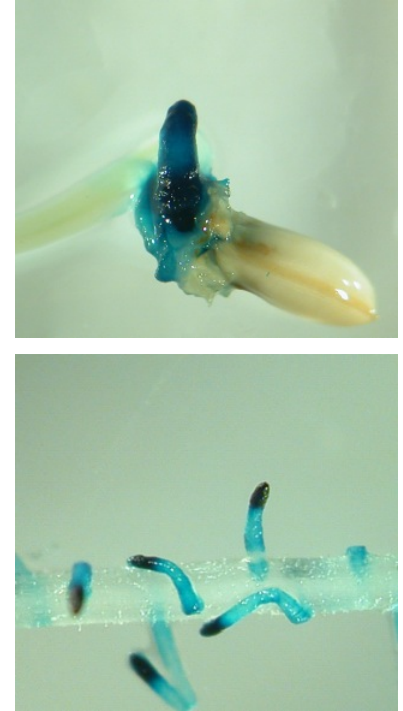
Oat



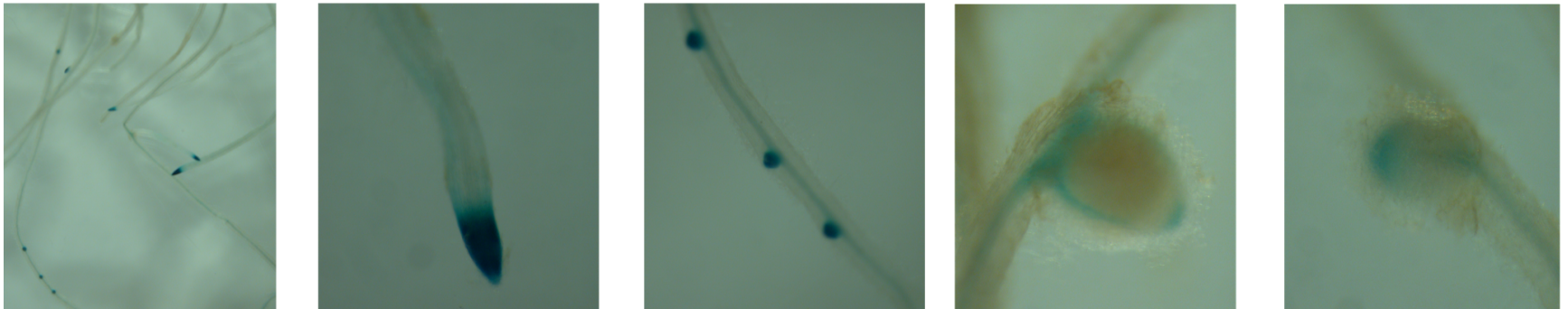
Arabidopsis



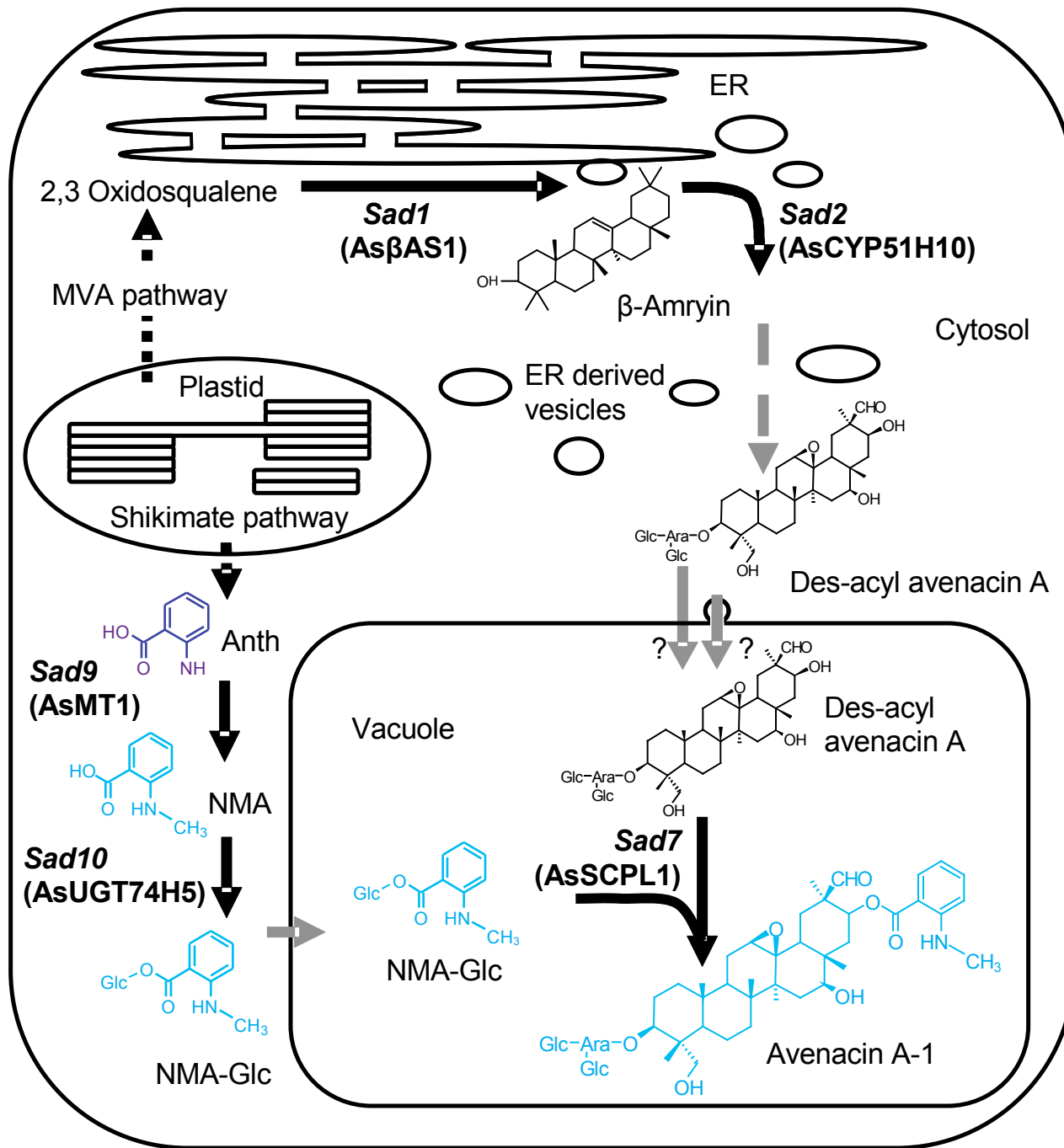
Rice



*Medicago truncatula*



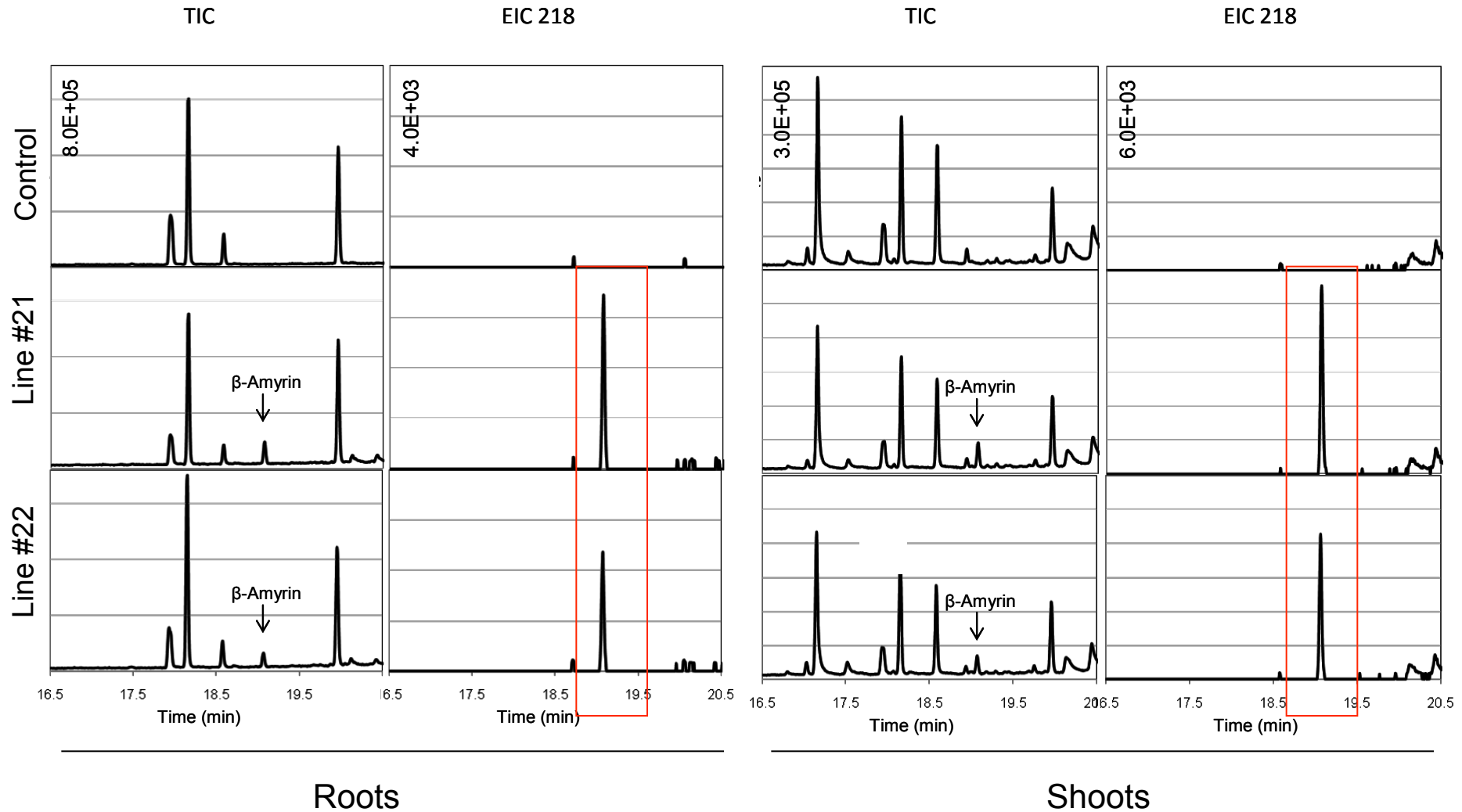




Owatworakit et al.  
JBC 2012

Mugford et al,  
Plant Cell 2013

# Expression of oat SAD1 in rice leads to $\beta$ -amyrin accumulation

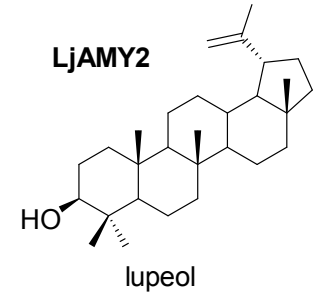
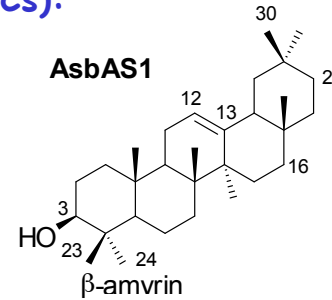


# Metabolic engineering for disease resistance

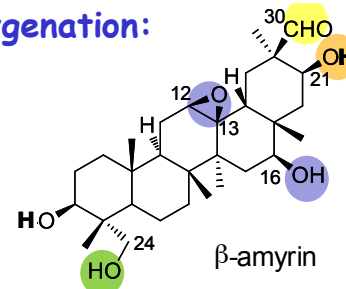


Take-all disease of wheat

## A) Scaffolds (OSCs):

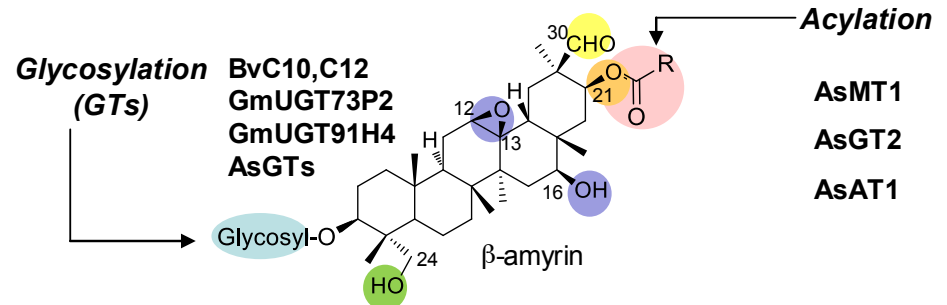


## B) Scaffold oxygenation: (CYP450s)

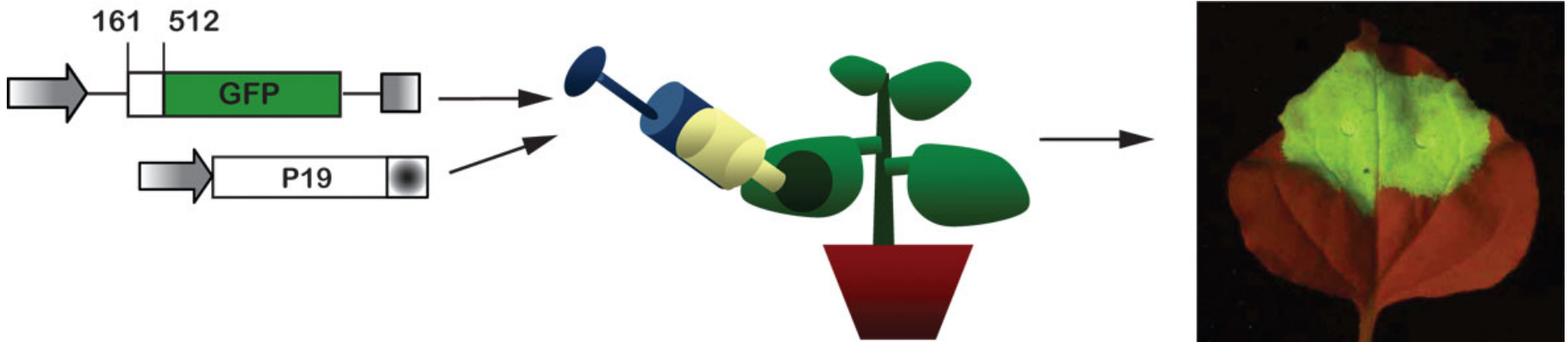


- AsCYPA
- AsCYPB
- GmCYP93E1
- GgCYP72A154

## C) Further modification:

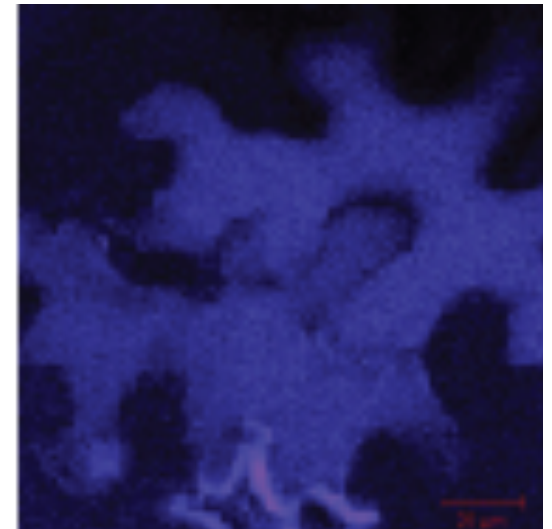
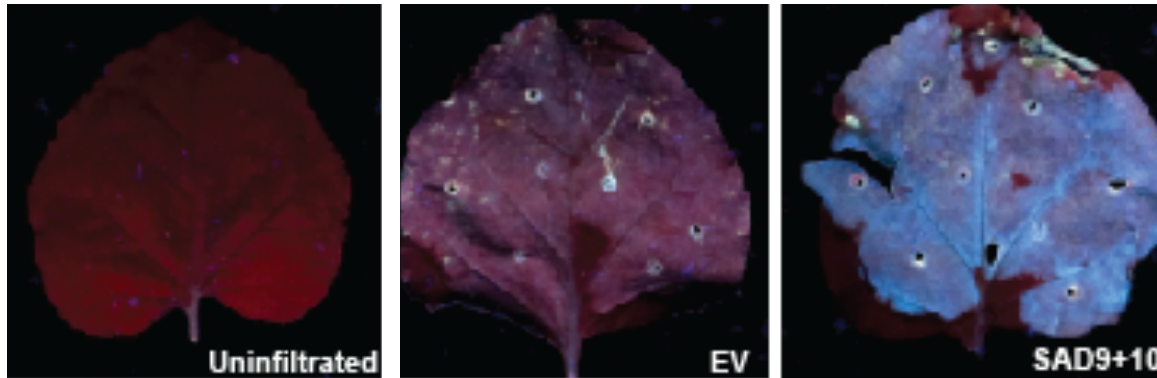


# HyperTrans - A virus-derived system for protein expression in plants



Sainsbury F and Lomonossoff G. (2008) *Plant Phys.* 148:1212

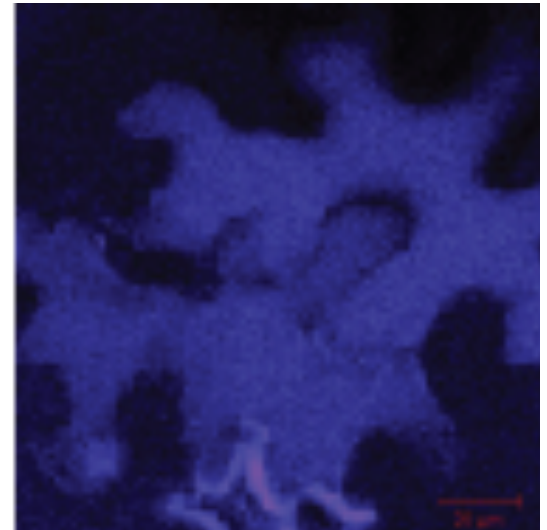
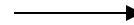
# Synthesis of the acyl donor N-methyl anthraniloyl-O-glucose



Accumulation of *N*-methyl anthraniloyl-*O*-glucose in the vacuole

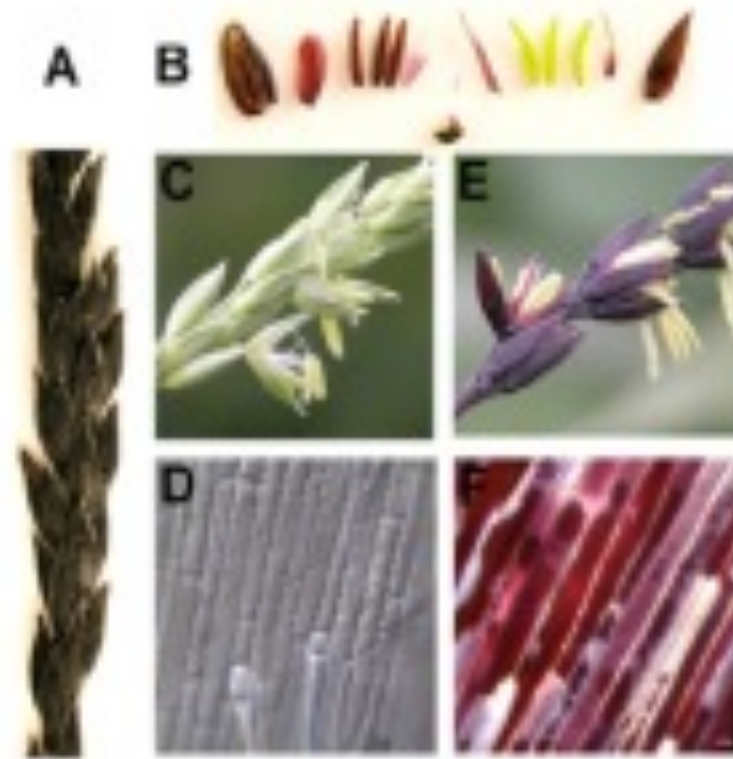
# Functional assembly of a terpene pathway - Proof of concept

TS  
+  
CYP450  
+  
CYP450  
+  
MT  
+  
GT  
+  
AT

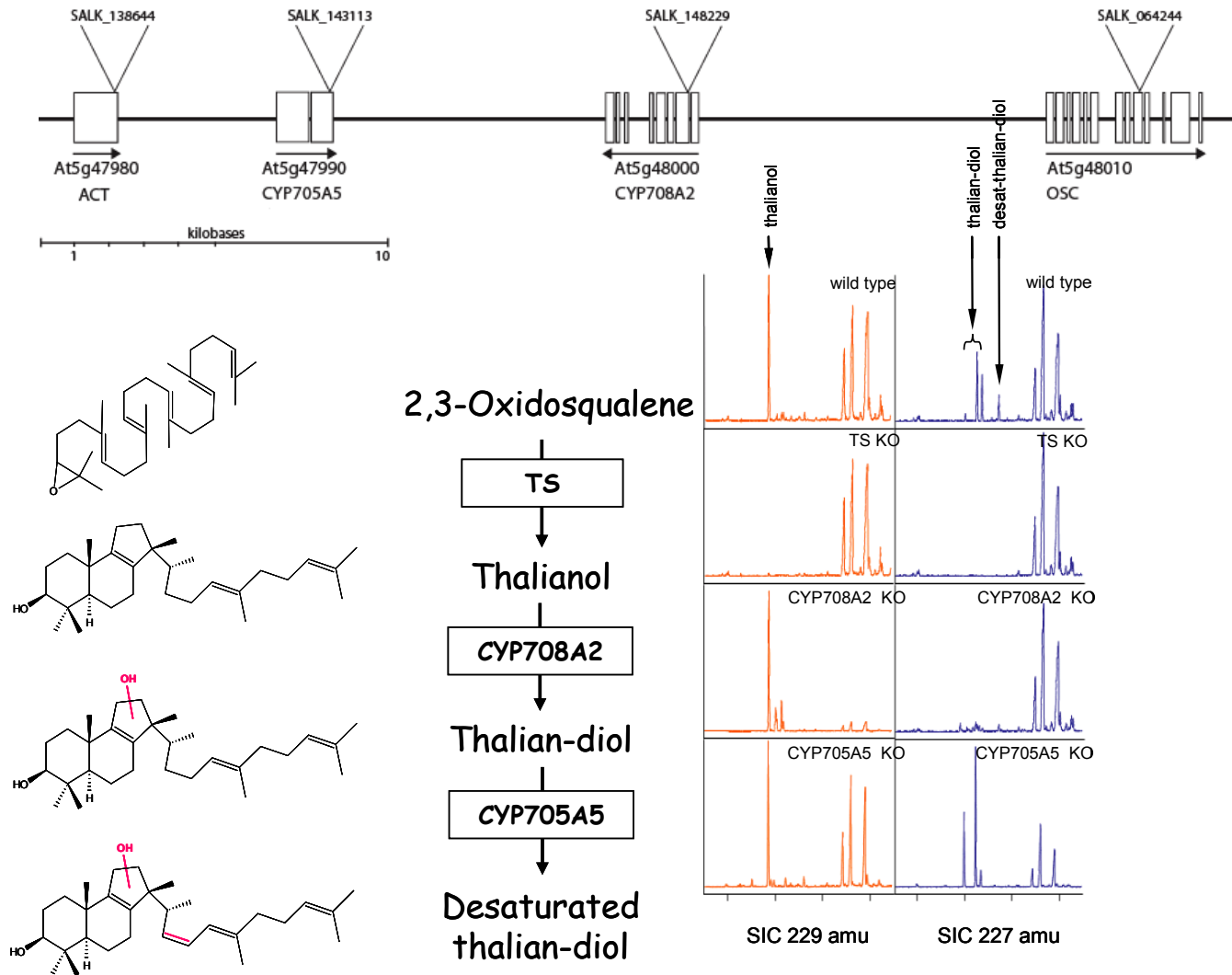


Accumulation of acylated  
terpene in the vacuole

# Anthocyanin synthesis - An example of a dispersed pathway

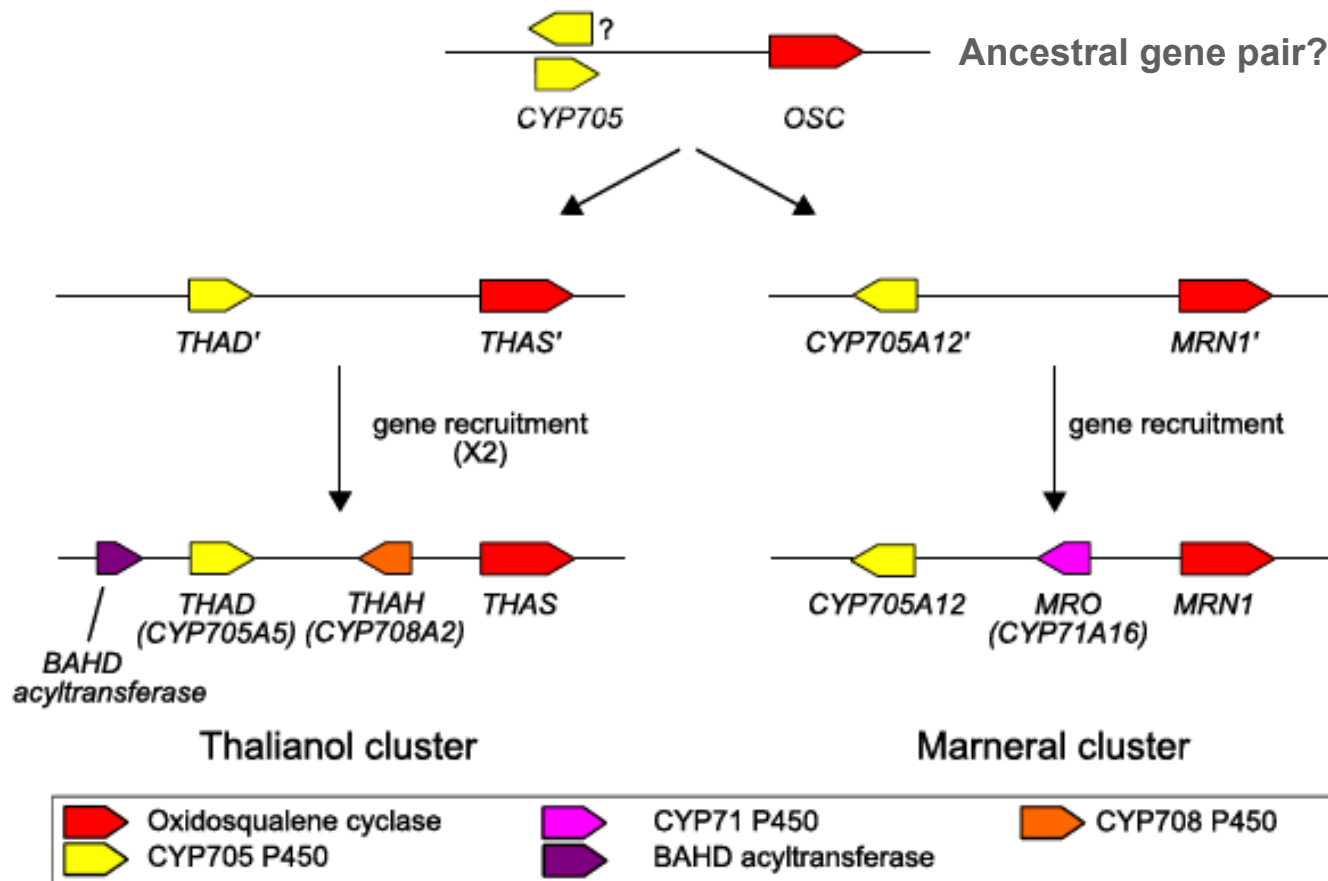


# The thalianol pathway - An example of another "operon-like" gene cluster



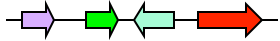


# Assembly of the thalianol and marneral gene clusters in *Arabidopsis*

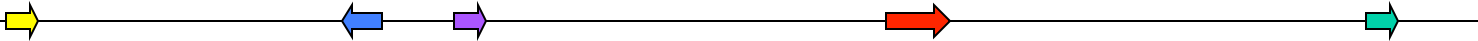


Chu et al. (2011) *Plant J* 66:66

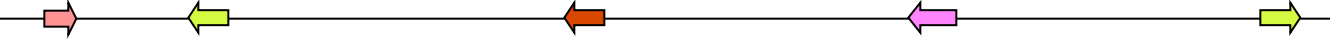
**Thalianol gene cluster:**  
*Arabidopsis thaliana* ( 35 kb)



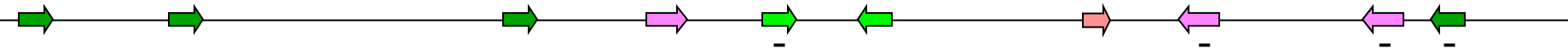
**Avenacin gene cluster:**  
Oat (*Avena strigosa*) ( 202 kb)



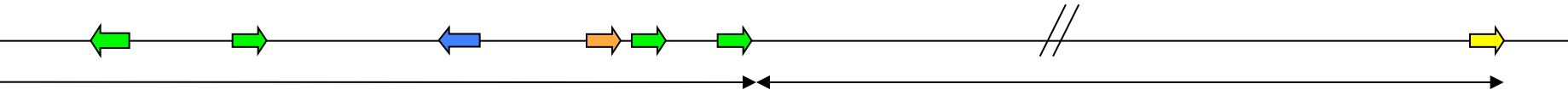
**Momilactone gene cluster:**  
Rice ( 170 kb)



**Phytocassane gene cluster:**  
Rice ( 245 kb)



**DIMBOA gene cluster:**  
Maize ( 15,618 kb)



554 kb

~15 Mb


Unlinked - Bx9

**Key: Signature enzymes:**

 Triterpene synthase

 Class I diterpene synthase

 Class II diterpene synthase

 Tryptophan synthase alpha homologue


**Tailoring enzymes:**


 CYP85


 CYP71


 CYP51

 CYP99


 CYP76

 BAHD acyltransferase

 Serine carboxypeptidase-like acyltransferase

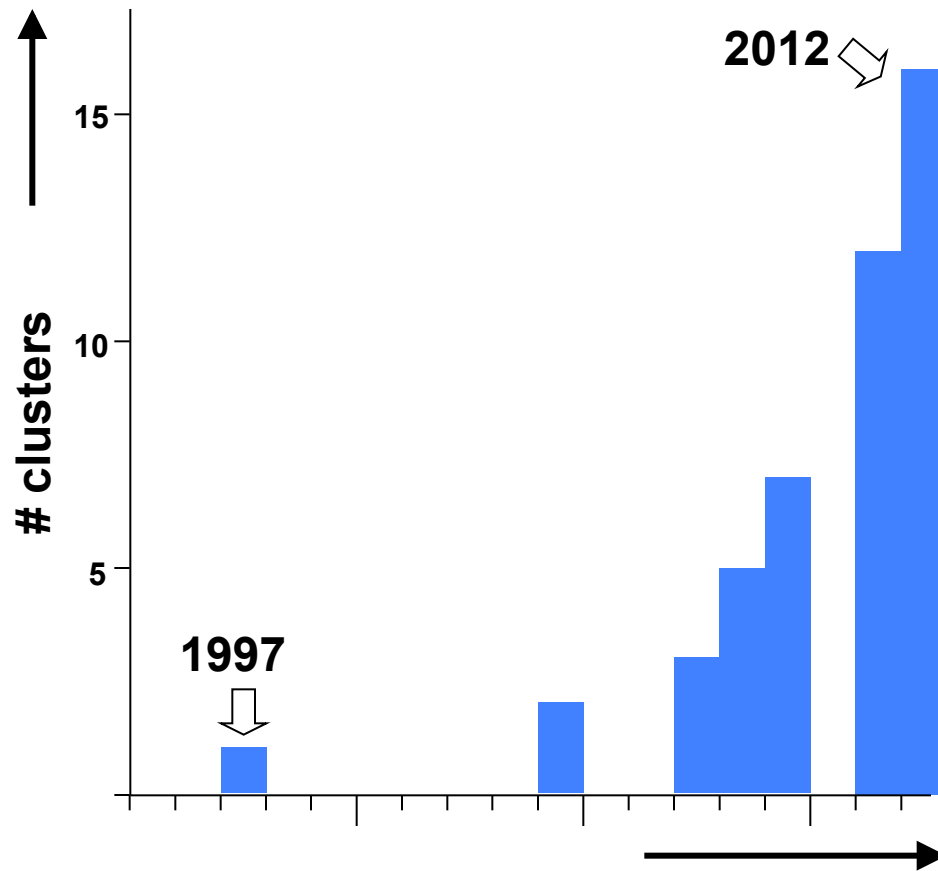
 Sugar transferase

 Methyltransferase

 Dehydrogenase



The number of examples of clustered secondary metabolic pathways is increasing



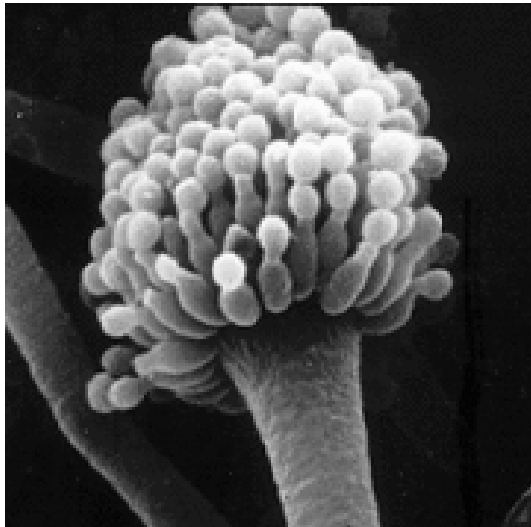
We currently only know about the genomic organisation of a handful of pathways

# Secondary metabolic gene clusters

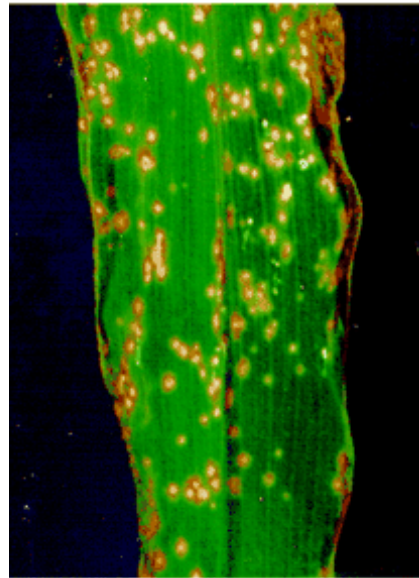
- Evolutionary toolkits for chemical innovation



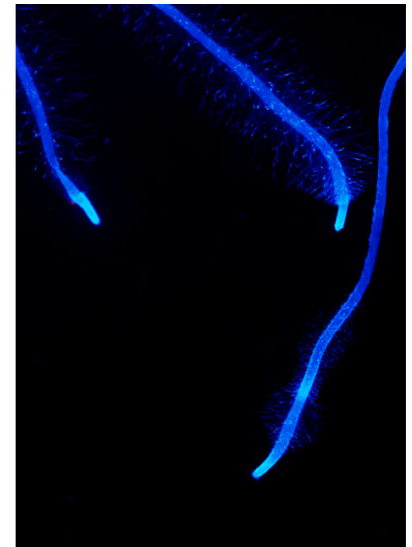
Antibiotic production - *Streptomyces*



Antibiotic/ toxin production -  
*Aspergillus*



Host-selective fungal toxins



Plant defence compounds

# Genome mining for pathway discovery

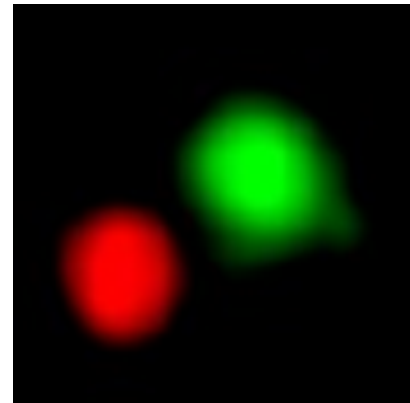
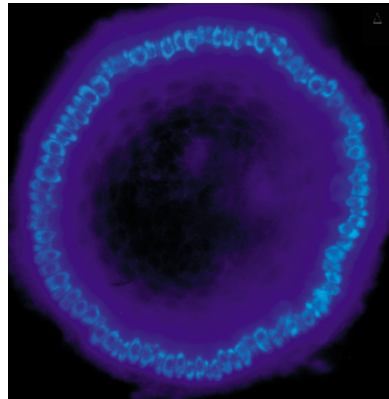


 **Signature enzyme**  
(makes scaffold)



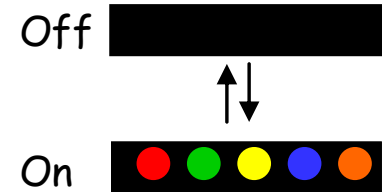
**Tailoring enzymes**  
(decorate scaffold)

Waking up 'silent' clusters:



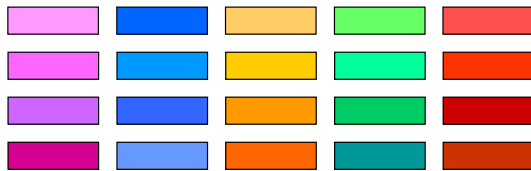
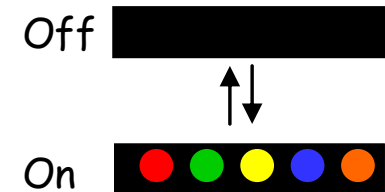
# Synbio - What metabolism has to offer:

- Co-regulated promoter sets



# Synbio - What metabolism has to offer:

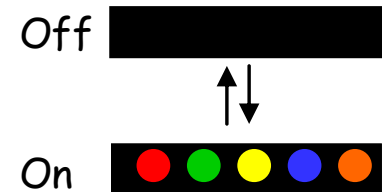
- Co-regulated promoter sets
- Modular pathway components



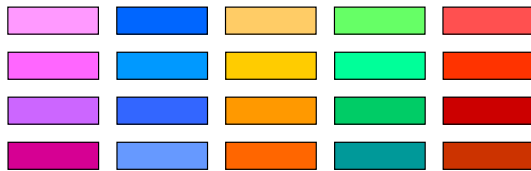


# Synbio - What metabolism has to offer:

- Co-regulated promoter sets



- Modular pathway components



- Refactored/synthetic clusters



‘SynTraits™  
,

## Osbourn Group:

Ramesh Thimappa  
Hans Neutzmann  
Aymeric Leveau  
Gemma Farres  
Thomas Louveau  
Athena Chu  
James Reed  
Rachel Melton  
Da Cheng Hao  
Mao Ye

Nan Yu  
Keiko Morikawa  
Tetsi Tsurushima

**SynBio postdoctoral  
position available  
NOW!**

## In collaboration with:

Rob Field, Martin Rejzek (JIC)  
George Lomonossoff, Frank Sainsbury (JIC)  
Andrew Hemmings (UEA)  
Lionel Hill, Alan Jones, Shirley Fairhurst (JIC)  
Soren Bak (Copenhagen)  
Kalliopi Papadopoulou (Univ. Thessaly)  
Luis Pena-Rodriguez (CICY, Mexico)  
Andy Greenland, Emma Wallingford (NIAB)  
Alex Boutanaev (Russian Academy of Sciences)  
David Nelson (Univ. Tennessee)

The Syntegron team: Susan Rosser (Univ. Glasgow),  
Jay Keasling (UC Berkeley), Paul Freemont (Imperial),  
Declan Bates (Univ. Exeter), Josh Leonard  
(Northwestern) and co-workers

Funding: BBSRC; EU FP7; EPSRC; TSB

