Genes - At the Centre of Plant Biology

Don Grierson
University of Nottingham
Norman Borlaug
1914 – 2009   Nobel Peace Laureate

Father of the
Green Revolution

Champion of
Resource-Poor
Farmers in the
Developing World

Founder of the World
Food Prize
The need for a series of new green revolutions

The world’s population is increasing from 6.8 to 9 billion by 2050.

We will need to produce more food in the next 50 years than we have in the previous 5,000 years!
Themes

Improved understanding enables us to produce food more suitable for our needs

Ethylene – A simple gaseous plant hormone with complex action

Molecular biology and genetic control of Tomato development and ripening
Ethylene effects seedling growth – The Triple Response

- \( \text{C}_2\text{H}_4 \)  
- \( +\text{C}_2\text{H}_4 \)

- Ethylene inhibits elongation growth (A)
- Causes radial swelling of the hypocotyl (B)
- Causes exaggerated hypocotyl hook formation (C)
- Inhibits root growth (D)
- Accelerate root hair formation
- Causes a general decrease in cell size

Arabidopsis and other Plants
Ethylene also causes Ripening

Mutants of ethylene action and ripening

Gr  R  Nr  rin  PG

rin  Green

Ripe  Nr
Prerelease of Tomato Genome Shotgun Sequence

The International Tomato Genome Sequencing Project prerelease version of the Tomato Genome Shotgun Sequence. December 1, 2009

Tomato Gene Microarrays GeneChip® Tomato Genome Array
Plants use ACC synthase and ACC oxidase to make ethylene.

**ENZYMIC STEPS CATALYSED BY**

- ACC Synthase (ACS)
- ACC Oxidase (ACO)

**SAM** (S-adenosyl Methionine) $\leftrightarrow$ ACC (1-amino cyclopropane 1-carboxylic acid) $\leftrightarrow$ Ethylene (Ethene)
Gene silencing inhibits ACO and Ethylene production during ripening.

Days from start of colour change

Ethylene production (nl/(g.hr))

Control

ACO-Antisense

(Hamilton et al., Nature, 1990)
Inhibiting ethylene synthesis by gene silencing in transgenic tomato plants

Inhibiting ACC oxidase (John et al 1995; Picton et al 1993)

Delays ripening and leaf senescence

Improves fruit shelf life
Altered ripening of low – ethylene melon by silencing ACO gene

Over- ripe melon
Antisense ACC Oxidase melon

HARVESTED 38 DAYS POST-POLLINATION
STORED AT 25ºc FOR 10 DAYS (J-C Pech Toulouse)
ACO1-Promoter-GUS Expression

Mature green

Breaker

Orange fruit

Ripe fruit
Expression of ACO1Prom-GUS in senescence, wounding, and infection

A Senescent leaf
B&C Young wounded leaf after 2h
D sprayed 5h with amino butyric acid
E&F Powdery mildew infection

(Blume & Grierson, 1997)
ACO and ACS gene expression in petals before and after pollination

(Llop-Tous et al, Plant Physiology 2000)
Gene Control of Ethylene Synthesis

- There are 9 ACS and 5-6 ACO genes.
- What do they all do?
- How are they all controlled?

- Complex transcriptional control(s)
- Control of protein turnover
In the fruit fly, the homeobox (Hox) genes control body plan (A). If these genes function properly the body structure is normal (B).

if the corresponding gene is mutated, the body structure will be changed.

For example, the Antennapedia (Antp) gene is normally expressed in thorax to control leg development, however, if it is expressed in the head the legs will form in the head (C).
Ethylene and Homeotic genes

• ACC Synthase and ACC Oxidase genes are regulated by homeotic genes

• This can change plant development
Ethylene synthesis and transcriptional control

SAM

↓

ACC

↓

ACC Synthase \((\text{LeACS2})\)

↓

ACC oxidase \((\text{LeACO1})\)

↓

Ethylene

Triple response

Ripening

Senescence, etc

Transcriptional control

RIN, a MADS box protein

\((\text{LeACS2 promoter})\)

(Ito et al., Plant J. 2008)

RIN, RIN

LeHB1, a homeobox protein

\((\text{LeACO1 promoter})\)

(Lin et al., Plant J. 2008)
LeHB-1 binds to LeACO1 promoter *in vitro*

A. Simplified structure of LeHB1

```
1                64               123         165               285
```

```
1 64 HD 123 Zip 165 285
```

B. Express the HD-Zip as GST fusion in yeast

```
33                                    176
```

```
33 GST HD Zip 176
```

C. *In vitro* binding assay with ACO1 promoter

```
-1855 F4-1 F3-1 F1-1 -1
```

```
-1855 F4-1 F3-1 F1-1 -1
```

(F1-1) (F4-1)

( Lin et al., Plant J. 2008)
Inhibiting LeHB1 expression by VIGS delays ripening

( Lin et al., Plant J. 2008)
Ectopic expression of LeHB-1 causes abnormal flowers and conversion of sepals into fruits.

(Lin et al., Plant J., 2008)
Multiple flowers within one sepal whorl
Ectopic expression of *LeHB-1* converts sepals into fruits

( Lin et al, Plant J. 2008)
But does ethylene really play a role in flower development?

- Perhaps it does if it is synthesised in specific cells at the right developmental stages…….?
Expression of ACS mRNA in developing melon flowers

Mfl = Male flowers
Hfl = hermaphrodite flowers
Ffl = Female flowers

Arrow heads show sites of mRNA accumulation

(Boualem et al, Science 321, 836-838 2008)
Homeotic transcriptional regulators act in specific cells to control flower development and fruit ripening.

What are the other genes in the regulatory network?

Which other genes in flowers and fruits are controlled by LeHB-1 and Ethylene?
How do the receptors work?
The mutant Nr ethylene receptor prevents ripening- Inhibition of Nr expression restores ripening

(Hackett et al., Plant Physiol., 2000)
Ethylene binds to receptors

Multiple receptors
Multiple CTRs
Additional interacting proteins
Many genes regulated by the signalling network

Ethylene signalling

Ethylene responses
Ripening etc

New genes expressed

Multiple CTRs

ER

TPR1

CTR1s

EIN2

RTE1

RAN1

MPK3/6

mRNA stability

Ubiquitin degradation

ERFs

EBF1/2

EIN3 EILs

Transcription

Cytoplasm

Nucleus

Ripening etc
Using biomolecular fluorescence complementation to investigate receptor-CTR interaction

Ethylene receptors
CTR protein
CTR C-terminal half of YFP
CTR protein
YFP fluorescence

Ethylene receptor NR + LeCTR3
Ethylene receptor NR + Negative control

Zhong et al., J Exp Bot 2008
Co-localisation of CFP- and YFP-tagged NR and LeCTR4 in the ER of Onion Epidermal Cells
Protein-protein interactions between Tomato ethylene receptors and LeCTRs

<table>
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<th>Receptors</th>
<th>CTRs</th>
<th>R1</th>
<th>R2</th>
<th>NR</th>
<th>R4</th>
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</table>
Over-expression of LeCTR2 increases hypersensitive response to infection.

Overexpressing the LeCTR2 N-Terminus

Wild type control
A new component SITPR1 interacts with ethylene receptors LeETR1 and NR -in yeast 2-hybrid and -in vitro experiments

It modulates ethylene signalling and auxin responses

(Lin et al, J. Exp. Bot. 59, 4271-4287, 2008)
Overexpression of SITPR1 in tomato (Solanum lycopersicon)

WT 3273A 3278A 3286A 3272A

Tran

Endo

rRNA

Images of tomato plants and果实.
Overexpression of SITPR1 results in parthenocarpic fruit and delayed abscission.
So what does that mean?
Models for TPR1 action

A

Air

+ C₂H₄

CTR1

TPR1

CTR1

receptors

Ethylene responses

LeIAA9 (down), SISAUR1-like (up)

Auxin responses

Epinasty, reduced apical dominance, Delayed abscission, Parthenocarpic fruit formation, Altered leaf and fruit morphology

B

Air

CTR1

TPR1

Reduced stature, epinasty, delayed or reduced reproduction, infertility
Our hypothesis is that TPR1 acts in the same fashion as the human TTC1 protein to compete with CTR1 for binding to the ethylene receptors, leading to CTR1 inactivation and failure to repress ethylene responses (A).

Alternatively, TPR1 interaction with the receptors causes their inactivation or degradation, also leading to enhanced ethylene responses (B).

Enhanced ethylene signalling caused by overexpression of TPR1 alters auxin responses through LeIAA9 and LeSAUR1-like genes. It is unclear whether or not TPR1 affects auxin signalling directly or indirectly.

(Lin et al., J Exp Bot, 2008)
Effects of Ethylene in Plants Depend on
-Multiple genes for biosynthesis, perception, and signalling, with complex regulation enables multiple actions

- Inhibition of shoot and root growth (triple response)
- control of flower development & organ identity
- flower and leaf senescence
- abscission
- fruit ripening
- response to biotic and abiotic stresses
- signalling to other pathways?
The Future

• ...It may be possible not only to control fruit quality and ripening

• ...but also to obtain flowers, fruits, and seeds without being restricted by the normal life cycle of the plant

Improved understanding enables us to produce food more suitable for our needs
TOMATO BIOTECHNOLOGY
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