UNDERSTANDING THE ROLE OF GIBBERELLIN SIGNALLING IN WHEAT ANTHER DEVELOPMENT DURING HEAT STRESS

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Introduction

1. Wheat is big business in Britain
- UK wheat production in 2014:
  - 16.6 million tonnes
  - 1.8 million hectares

2. Climate change will impact yields
- Wheat growth cycle will shift to avoid terminal drought
- BUT
- More extreme pre-anthesis heat waves threaten yields

3. Heat stress reduces male fertility
- Temperatures ≥30°C inhibit formation of viable pollen
  - Due to disruption of anther tissue life

4. Male fertility regulated by Gibberellin signalling
- Plant hormone GA required in cereals for anther formation and development
- GA signalling in modern wheat has been altered

Semenov et al., (2014)
HT disrupts anther development

- High temperatures during booting reduces yield
- Due to effects on pollen development
  - PMC Meiosis
  - Tapetum programmed cell death
  - Carbohydrate metabolism
  - Anther dehiscence

Saini et al., (1983)
Staging anther development
Staging anther development
Gibberellin signalling is crucial for anther development

GA

GA2ox

GA3ox

GA20ox

GA3ox

GA

GID1

DELLA

DELLA

DELLA

DELLA

DELLA

GA

GID1

GID1

SCF

Ub

Ub

Growth Responses

GA20ox

GA3ox

GA20ox

GA3ox

GA

GID1

GID1

SCF

Ub

Ub

Growth Responses

SE

SE

GA

GID1

GID1

SCF

Ub

Ub

AP25

AP37

Cp1

C6

ADF

CYP703A3

KAR

Tapetum Programme

Cell Death

Ubisch body and exine formation

Carbohydrate mobilisation?

miR159

SLR1

DELLA

GAMYB

TIP2

TDR

EAT1 (bHLH141)

UDT1

Growth

Responses

26S

Proteasome
Gibberellin signalling is crucial for anther development

- GA-biosynthesis and signalling required for normal anther formation and development
- DELLA regulated GAMYB controls tapetum PCD

Aya et al., (2009)
Do *Rht-1* alleles influence anther response to HT?

“Green Revolution” genes:

Pleiotropic effects:

- Grain size
- Seedling Vigour
- Male fertility?
Does HT disrupt GAMYB and bHLH141 function?

*TabHLH141:*
- Tapetum PCD executor
- Possible DELLA interaction
- Possible GA regulation

*Nui et al., 2013*
Does HT disrupt GAMYB and bHLH141 function?

An experiment to find out:
- *Rht-B1* Near Isogenic Lines grown in CE, cv. April Bearded
- Staging used to target 3 day HT (34 °C) to prominent tapetum stage
- Sampling after 24h and 72h
- Thin section microscopy to assess physiological damage
- qRT-PCR analysis of *TabHLH141* and *TaGAMYB* expression
HT causes premature tapetum PCD in wheat

$T_0 \ 20^\circ C$
Stage 4 – Tetrads
*Rht-B1a*

$T_1$
+24 Hours

$T_2$
+72 Hours

20 °C

34 °C
Preliminary qPCR results

- *TaGAMYB* and *TabHLH141* upregulated during observed stages

- *TaGAMYB* not affected by HT in *Rht-B1a* background

- HT increases expression of *TabHLH141* in *Rht-B1a* and *Rht-B1b* lines

- Reduced expression in *Rht-B1b* background would fit GA-mediated model

- Could contribute to accelerated PCD
What’s next?

- Characterise function *TabHLH141* and *TaGAMYB* in wheat anthers

- RNAi $T_0$ lines genotyped. Selection of $T_1$ lines to commence shortly
- *GAMYB::SRDX* construct awaiting transformation
- *gamyb* TILLInG mutants obtained. Crossing for stable double/triple lines
What’s next?

- Understand impact of HT on anther hormone profile and transcriptome
  - Quantification of important hormones (GA, ABA, IAA, JA)
  - RNAseq transcriptome analysis
What’s next?

- Develop wheat anther GA-signalling network
- Protein-protein interaction studies (*Rht-1, bHLH141, GAMYB et al.*,)
- RNAseq and qPCR expression profiling

20 mM 3-AT

1. Empty bait / TabHLH141
2. TabHLH141 / Empty prey
3. Rht-D1 GRAS / TabHLH141
4. AtGAI/TabHLH141
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