Characterisation of rye-grass (*Lolium* sp.) transcriptome-wide response to ALS-inhibiting herbicides

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Background

- Non-target-site-resistance (NTSR) to herbicides is a major cause for failure of the chemical control of grass weeds (Weed Res 51: 433-437 (2011))

- Several gene families shown to be involved in NTSR:
  - cytochromes p450
  - glutathione-S-transferases
  - glycosyl-transferases
  - ABC transporters

- But the genetic determinants of NTSR are still poorly known to date

- Hypothesis: NTSR endowed by
  - changes in the regulation of a range of genes
  - mutations in the coding sequence of a range of genes

- NTSR is part of the response of weeds to herbicides
Objectives

- Characterise the **processes involved** in response to *herbicides inhibiting ALS* in rye-grasses using a transcriptome sequencing approach.

- **Identify gene functions/families** involved in herbicide response and potentially involved in NTSR.

- **Identify transcripts potentially involved** in NTSR.
Material & Methods

- Comparative whole-transcriptome sequencing (RNA-seq) of 3 R and 3 S rye-grasses from the same field population before and after the application of pyroxsulam:

  - Cloning
    - one plant with X tillers
    - X clones with one tiller

  - Pools of 3 resistant plants
  - Pools of 3 sensitive plants

  - RNA Full transcriptome sequencing (Illumina)

  - Transcriptome assembly
    - Annotation
    - Read mapping

  - Validation of expression levels by RTqPCR
    - Normalization with CAP, UBQ & GAPDH (Plos One 8: e63376 (2013))

  - Candidate NTSR genes
Transcriptome sequency and assembly: LOLbase

- 323,833,502 raw 100-base reads

- **LOLbase = 92,381 transcripts**
  - N50 = 771 nucleotides
  - total base count = 91,557,401 nucleotides

- **LOLbase = 81,663 predicted peptides**
  - 57% Pfam annotation
  - 36% GO annotation
  - average length = 177 amino acids

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**Graph:**

- Number of genes per Mb of genome
- Size genome (Mb)
- R² = 0.9598

- Genomes:
  - *O. sativa*
  - *B. distachyon*
  - *S. bicolor*
  - *Z. mays*
  - *H. vulgare*
LOLbase expression data

- **Good correlation** between expression levels from RNA-seq and from RTqPCR – 19 transcripts tested
General response to ALS inhibitors

- 16,727 transcripts (18.2%) up-regulated & 23,731 transcripts (25.8%) down-regulated at least 3-fold by the application of pyroxsulam

- Identification of **GO terms enriched in up-regulated & down-regulated transcripts** after herbicide application compared to UT (p-value<10\(^{-10}\)):
  - monooxygenase activity, iron ion binding (**P450s**)
  - transferring hexosyl groups (**GTs**)
  - ATPase activity (**ABC transporters**)
  - multidrug transport (**MatE**)
  - **GSTs** not assigned a GO term

  → enriched in up-regulated transcripts, some specific to R or S

- photosynthesis, mitosis & nucleus components enriched in down-regulated transcripts
  → turned off
R & S specific responses

- 50,128 transcripts (54%) differentially expressed between 7 R pools & 7 S pools at at least one time point

- Differential expression of Pfam families assumed to be involved in NTSR
  - up-regulated in R:
    - from 28 transcripts (ABC transporters) to 127 transcripts (UDPGT)
    - peak at 12HAT for P450s (76), UDPGTs (69), GTs (31), GSTs (31)
  - more transcripts up-regulated in S for P450s, UDPGTs, ABC transporters, and GSTs
Identification of candidate NTSR transcripts

1) Up-regulated at least 3-fold in R UT/S UT

2) Up-regulation maintained after herbicide application

3) Up-regulated in R HAT/ R UT (facultative)

4) Belonging to gene families involved in NTSR

→ 30 candidates tested: 6 P450s, 6 GSTs, 1 GT

→ measure of the expression by RTqPCR in 124 untreated plants samples (R or S to 2 different herbicides: pyroxsulam & iodo+meso) from 10 rye grass field populations
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2 P450s and 1 GT up-regulated in R UT

1 GST up-regulated in R of populations with most R frequency
Identification of candidates for NTSR

Pyroxsulam (log10)

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iodosulfuron + mesosulfuron (log10)

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Expression levels in 124 plants

- Expression patterns of the two P450s correlated

- No correlation for other pairwise expression levels → different mechanisms/regulation patterns

- No clear cut correlation between the plant phenotype and the expression levels of the 4 genes

![Expression levels graphs for CYP72A, CYP81B1, GTA, GSTA]

- Plants resistant to both herbicides
- Plants resistant to one herbicide, sensitive to the other
- Plants sensitive to both herbicides
Linear Discriminant Analysis

- test whether the expression levels can **discriminate the plant phenotypes** (R vs S)
- find the **linear combination** that optimises the discrimination

→ combinated relative expression level (CREL) of the 4 genes tested

\[
\text{CREL} = 1.04 \times CYP72A + 0.14 \times CYP81B1 + 1.54 \times GTA - 0.28 \times GSTA
\]
CREL values differentiate NTSR individuals...

- Better R/S discrimination when considering both herbicides
- CREL > 2 ↔ plant resistant to at least one herbicide

... But not all NTSR individuals
1) LOLbase is a resource of interest to study NTSR and identify NTSR genes

2) Strong variability in candidate gene expression among NTSR plants
   → Diversity of NTSR mechanisms

3) Using a combination of the expression of several genes improves the discrimination between resistant and sensitive plants
   → NTSR is a polygenic trait

4) Considering R to several herbicides improves discrimination of NTSR plants
   → The candidate genes identified may be involved in NTSR to both pyroxsulam and iodosulfuron+mesosulfuron