Bioengineering: A potential new tool for white rot control

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Crop & Food Research, NZ
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Overview

- Strategy
- Recap of position on Jan 2007
- 2007-2008 progress
- 2008 – 2010 research
Strategy

- Other species have gene sequences that produce enzymes which can neutralize the white rot toxin oxalic acid (oxalate).
  - Oxalate oxidase (oxox)
  - Oxalate decarboxylase (oxdc)
Strategy

- Bisected young wheat root showing oxalate oxidase activity (dark strips)
- Natural defence mechanism

- *S. sclerotinia* mutants for oxalic acid production cannot penetrate plant cells
Researchers have introduced the genes into:

- Lettuce (2006)
- Peanut (2005)
- And previously tobacco, poplar, sunflower, soybean, and oilseed.
- All publications showed degrees (up to 100%) of increased fungal resistance.
Strategy

It is now possible to move genes between species

- Need improved garlic transformation system
Recap – Research History - 2002-06

- Expressed wheat oxox in onion root
- Produced two functional transgenic onion lines
- Initial assays indicated the oxalate oxidase gene was functional
Graph of lesion length after *S. cepivorum* challenge on transgenic magainin and oxalate oxidase expressing onion roots

![Graph of lesion length after S. cepivorum challenge on transgenic magainin and oxalate oxidase expressing onion roots](image-url)
Recap – GORAB involvement

- In 2006 funded proliferation of two existing (antifungal) transgenic lines

- By Jan 2007 seed from one line was produced and multiple bulbs obtained from the other
- Also in 2006 funded gaining access to improved antifungal gene sequences

- By Jan 2007 we had obtained access to barley oxox cDNA sequence from Syngenta and a understanding on how to proceed should the technology work.

- Also identified the oxdc gene from Flammulina as potentially a even better gene to confer AWR resistance
- In 2006 funded development of an efficient garlic transformation system
- By Jan 2007 high frequency shoot regeneration was achieved
GORAB 2007-2008 three objectives

1# Complete garlic transformation research
2# Evaluate $F_1$ generation antifungal onions
3# Develop improved antifungal constructs for transformation
1# Garlic transformation

1-14 transgenic lines

Non-transgenic

Plasmid controls
Garlic transformation

- Plants have been regenerated
  - 34 different events regenerated from 3 experiments
  - ~100x improvement over previous garlic transformation systems
2# Evaluation of F₁ generation antifungal onions
Two antifungal lines for analysis

Lines contained a wheat oxox gene which produces the enzyme oxalate oxidase which can inactivate the AWR fungal toxin oxalic acid

- Line 04.19 Only formed 5 bulbs, no flowers
- 4/5 bulbs died during storage
- 1/5 bulbs grew again and has now formed a larger bulb
- Taken samples from this for molecular analysis
The second line 0407.46 produced lots of seed

~100 seed was germinated and samples analysed for inheritance of the transgene

Transgene was inherited as expected in a 3:1 ratio (26:10)
Evaluation of line 0407.46

Seedlings were grown up in hydroponic tubs so that root samples could be taken and analysed.
Evaluation of oxalate oxidase activity

\[
\text{oxalate} \quad \xrightarrow{\text{Oxalate oxidase}} \quad 2\text{CO}_2 + \text{H}_2\text{O}_2
\]

Purple stain

4CN
Initial results on seedling roots

[Images of seedling roots with +ve and -ve annotations]
Older root tissue – results are inconclusive
Developing an improved quantitative assay

- Amplex Red Quantitative assay
- Remove endogenous substrates
- In gel staining
- Dot blot assays
Additional analysis

We have also frozen root samples ready for:

- Northern analysis to check the presence of the oxox mRNA transcript
- Western analysis to check enzyme activity

- We also have ~50 bulbs in storage ready for sprouting for further analysis and challenge with OWR pre-germinated spores
3# Development of improved antifungal constructs for transformation
Two new constructs have been created - why?

- Problem: - Original wheat oxox sequence reported to be glycosylated differently in transgenic plants – affecting performance
- Expression in first lines is still under investigation
- Improved oxox (barley) cDNA sequence is available from Syngenta
- A oxdc sequence reported by Dias et al. (2006) conferred 100% resistance in lettuce
DNA constructs

GER1a with onion codon usage

711 bp
CaMV35s – TMVΩ – bOxOxonion - Ocs

CaMV35s – TMVΩ – OxDconion - Ocs
Current status

- Constructs have been inserted into tobacco and we are currently regenerating tissue from this.
- We are ready to use these constructs on garlic using our garlic transformation system.
2007/2008 Research Proposal

for the

California Garlic and Onion Research Advisory Board

Assessment of a biotechnology approach for creating onion white rot tolerant germplasm

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### Future research 2008/9

#### Timeline

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*future obj.*
Acknowledgements

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