CROP & FOOD RESEARCH
Mana Kai Rangahau

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

Strategy

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.

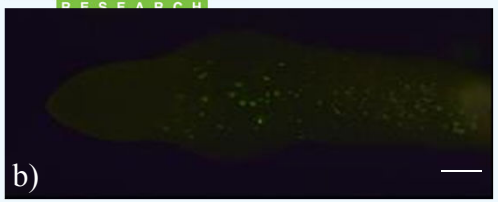
Strategy

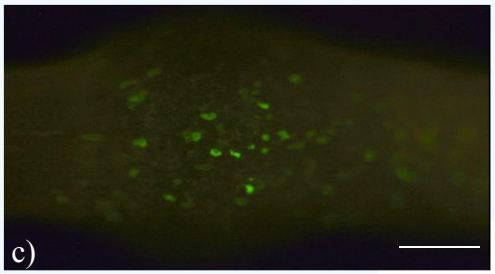
It is now possible to move genes between species

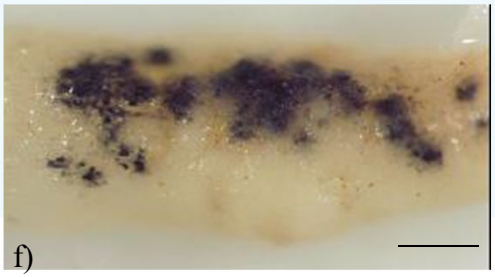
- Need improved garlic transformation system

Recap – Research History - 2002-06

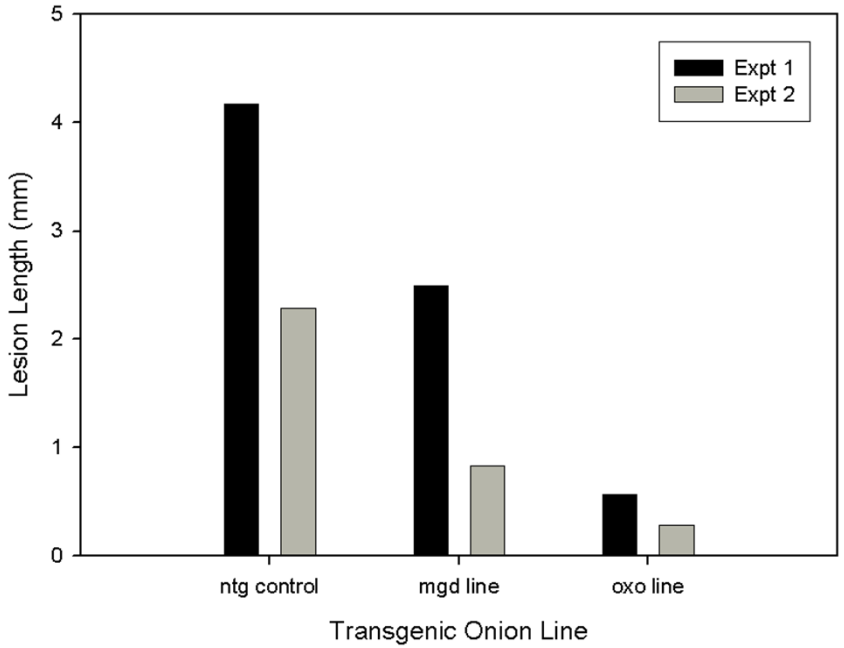
- Hunger, Sarah (2007) – The development and assessment of onion germplasm engineered to resist *Allium* White Rot attack. Ph.D thesis Lincoln University
- Expressed wheat *oxox* in onion root
- Produced two functional transgenic onion lines
- Initial assays indicated the oxalate oxidase gene was functional







Graph of lesion length after *S. cepivorum* challenge
on transgenic magainin and oxalate oxidase expressing onion roots



Recap – GORAB involvement

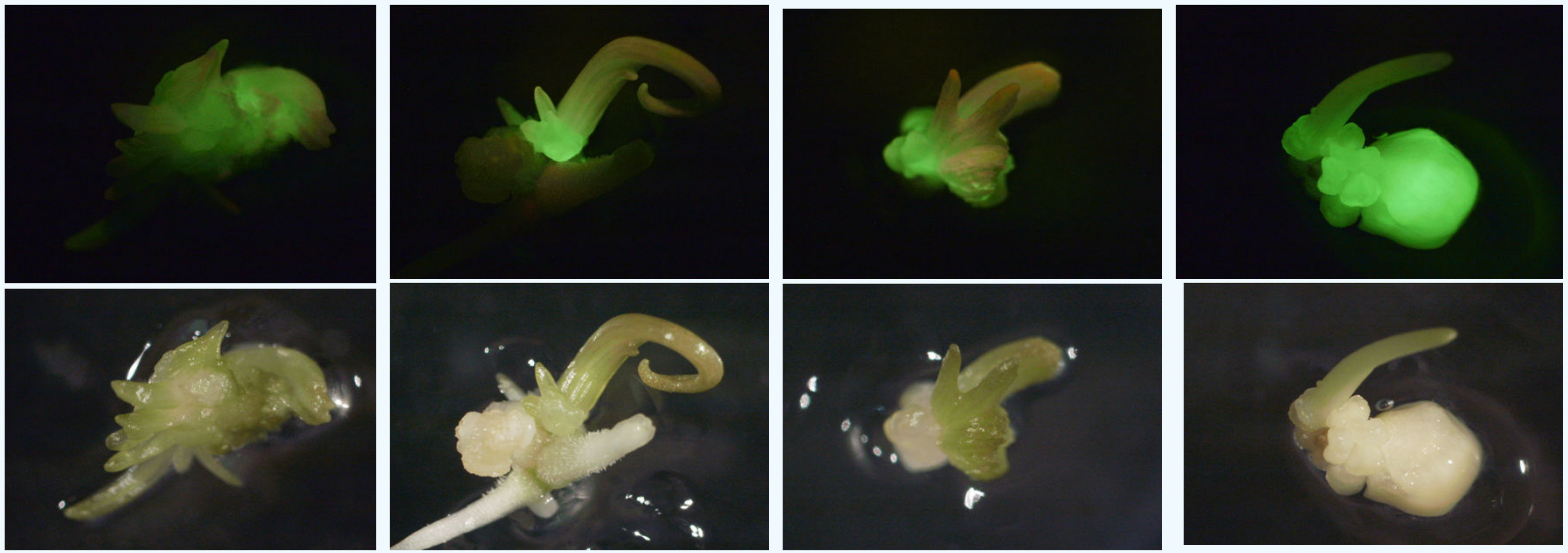
- In 2006 funded proliferation of two existing (antifungal) transgenic lines

- 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



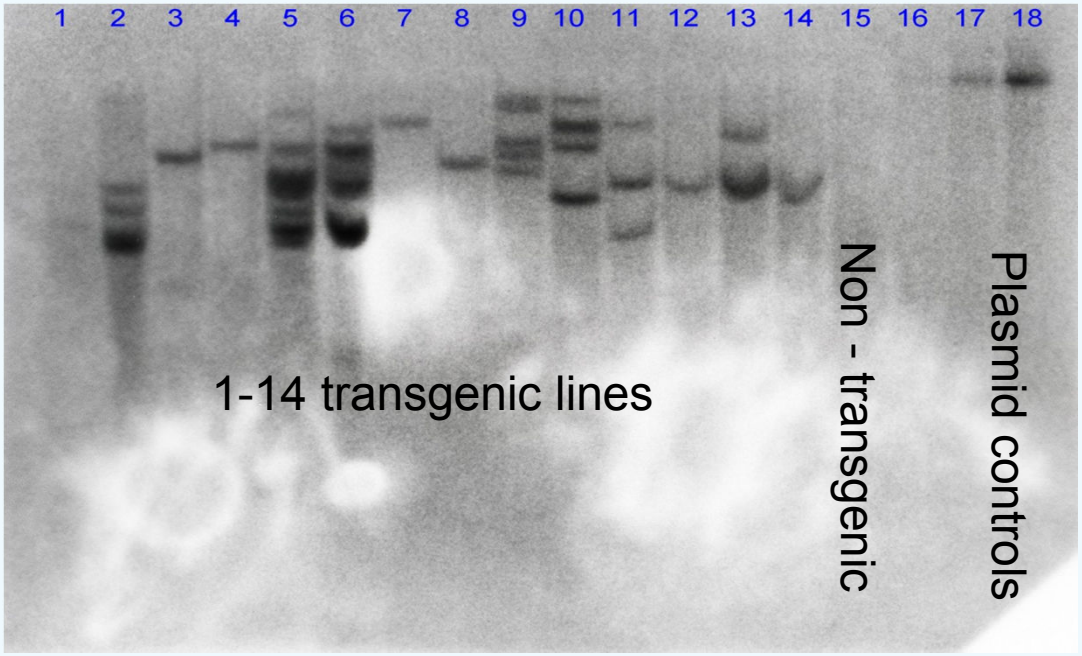
GORAB 2007-2008 three objectives

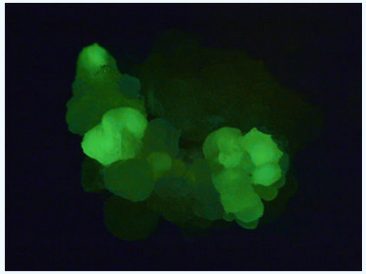
1# Complete garlic transformation research

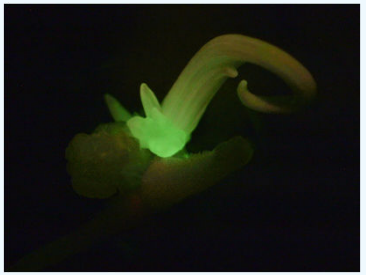
2# Evaluate F_1 generation antifungal onions

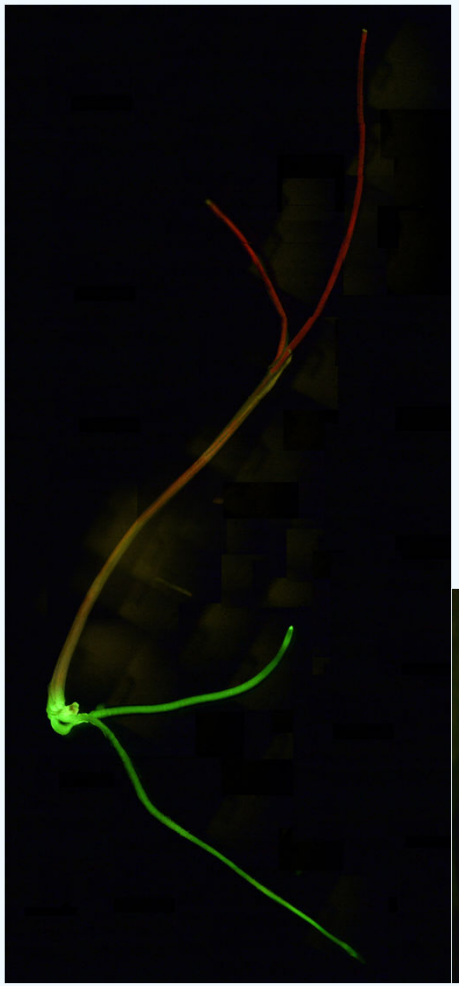
3# Develop improved antifungal constructs for transformation

1# Garlic transformation



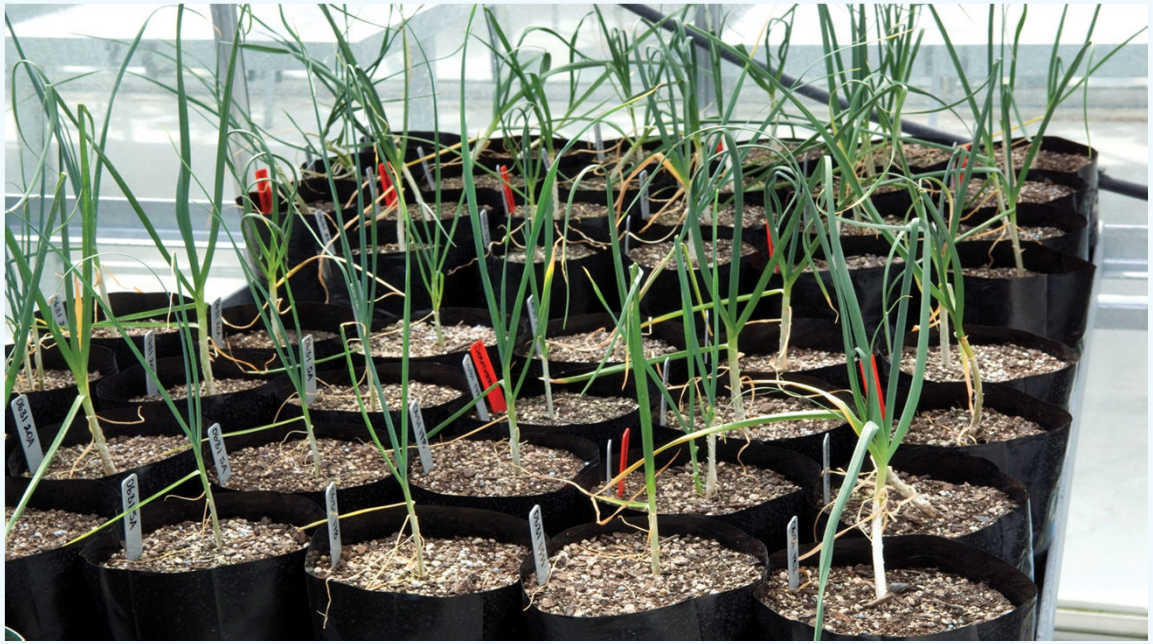






Garlic transformation

- Plants have been regenerated
  - 34 different events regenerated from 3 experiments



  - ~100x improvement over previous garlic transformation systems

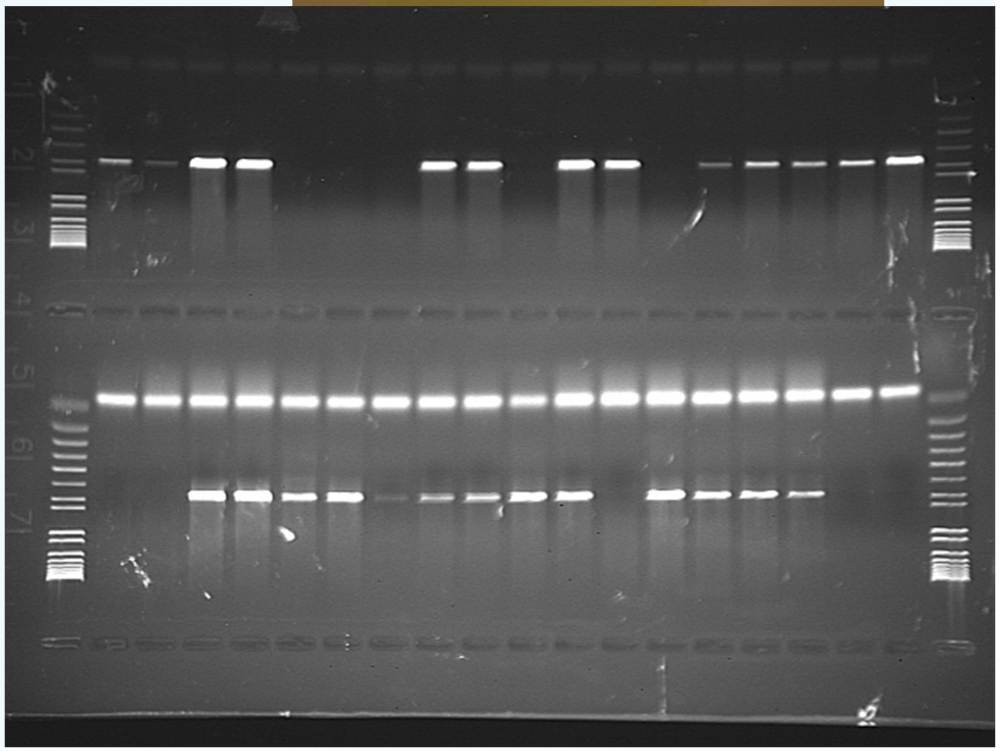
2# Evaluation of F_1 generation antifungal onions

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

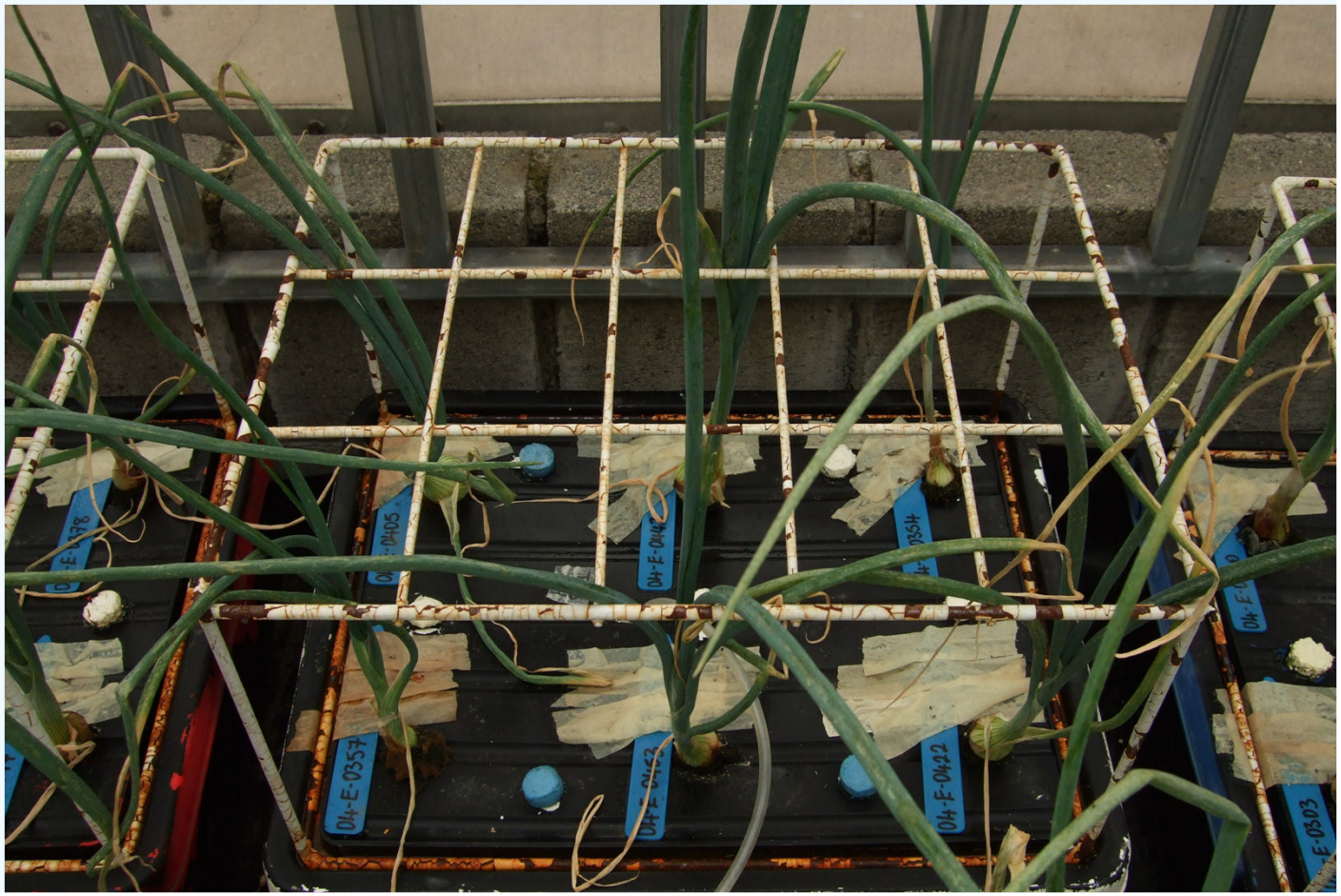
- Line 04.19 Only formed 5 bulbs, no flowers
- 4/5 bulbs died during storage
- 1/5 bulbs grew again and has now formed a larger bulb
- Taken samples from this for molecular analysis

- 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)

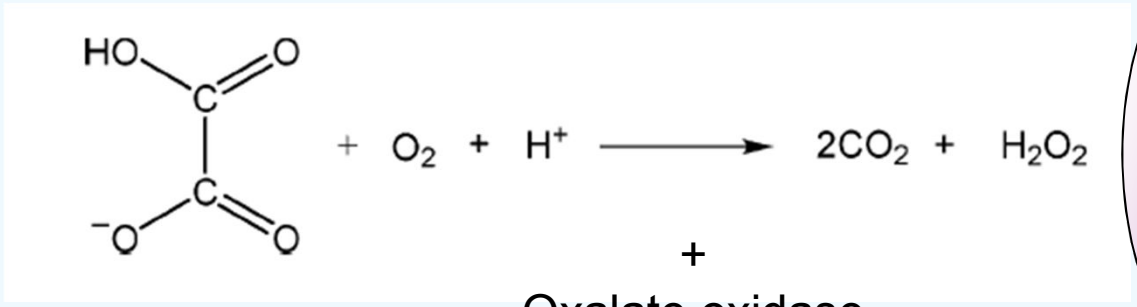


Evaluation of line 0407.46

Seedlings were grown up in hydroponic tubs so that root samples could be taken and analysed



Evaluation of oxalate oxidase activity

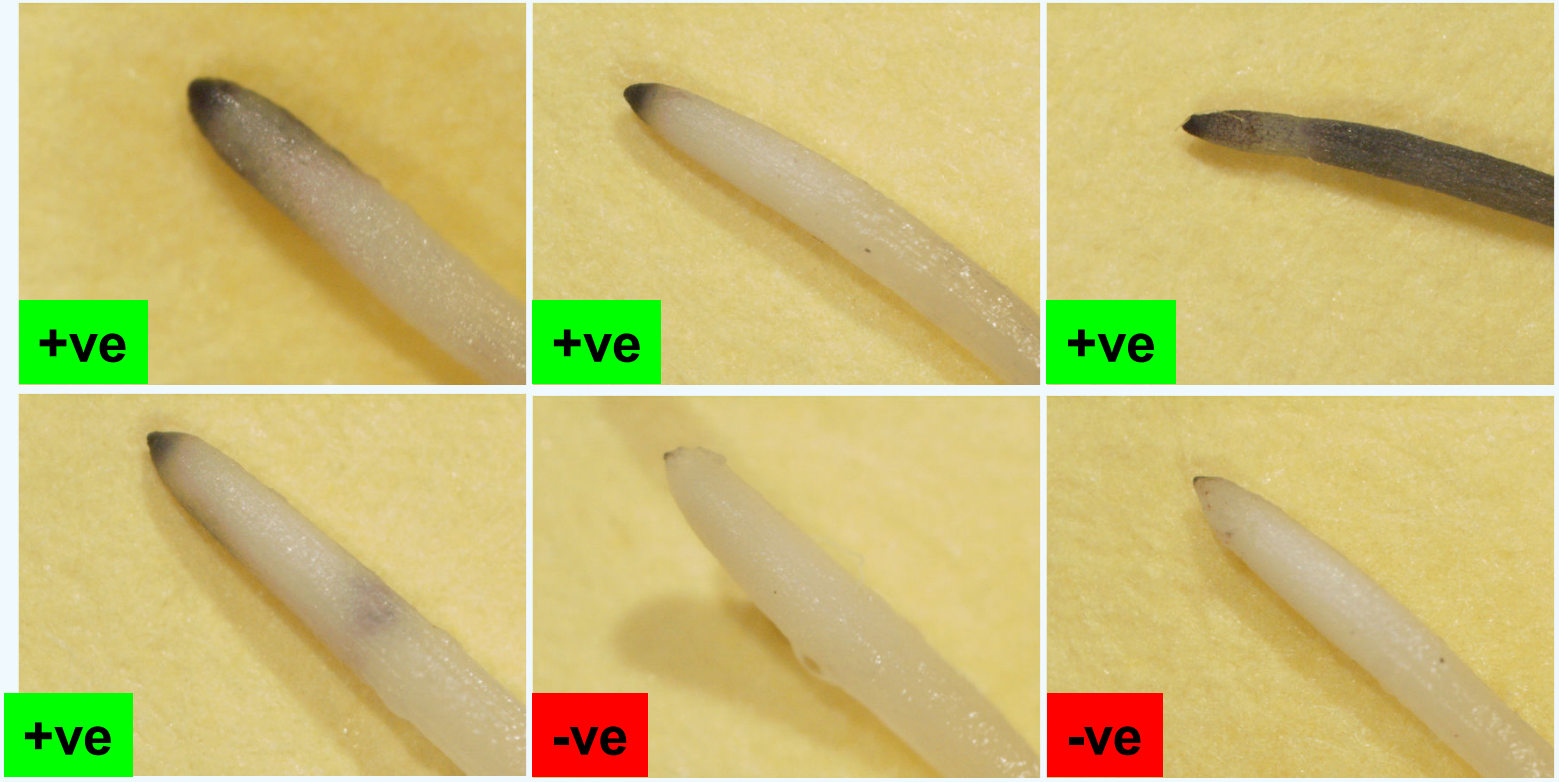


oxalate

4CN

Purple stain

Initial results on seedling roots



Older root tissue – results are inconclusive

-ve

+ve

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

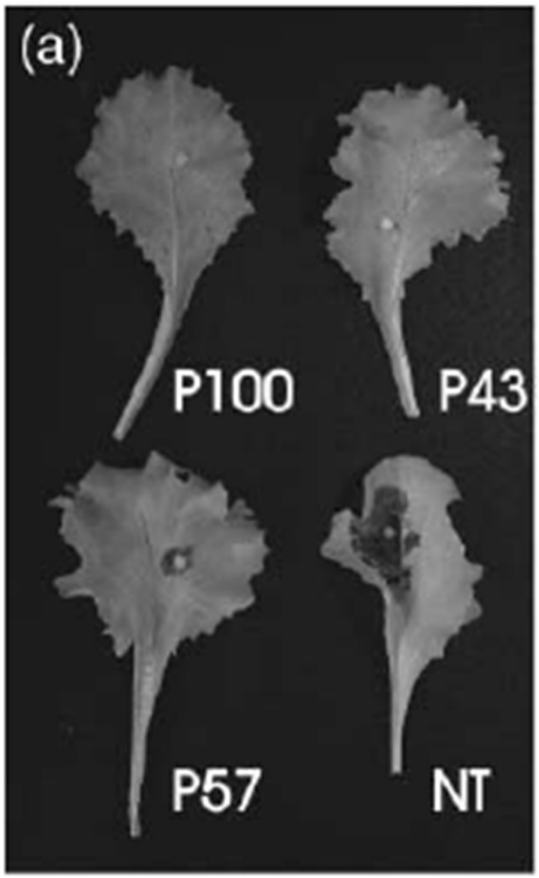
- We also have ~50 bulbs in storage ready for sprouting for further analysis and challenge with OWR pre-germinated spores

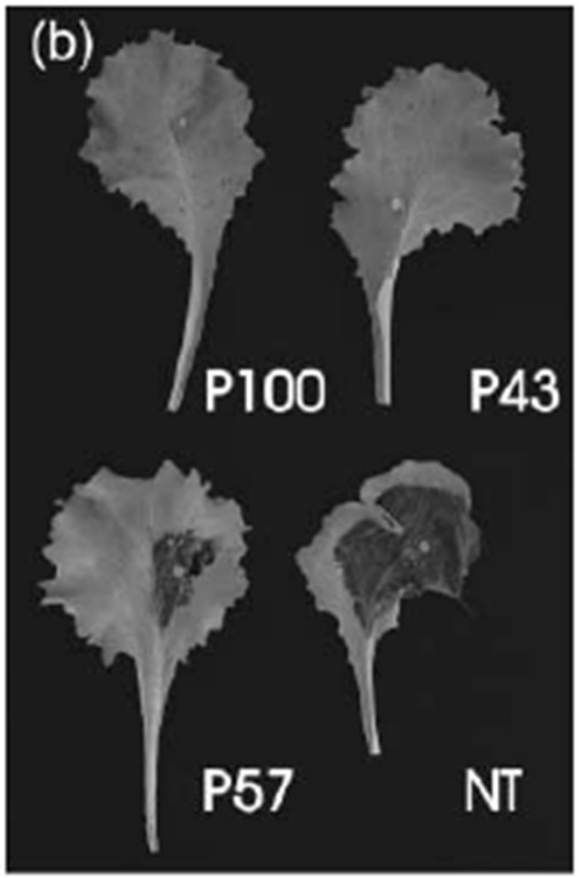
3# Development of improved antifungal constructs for transformation

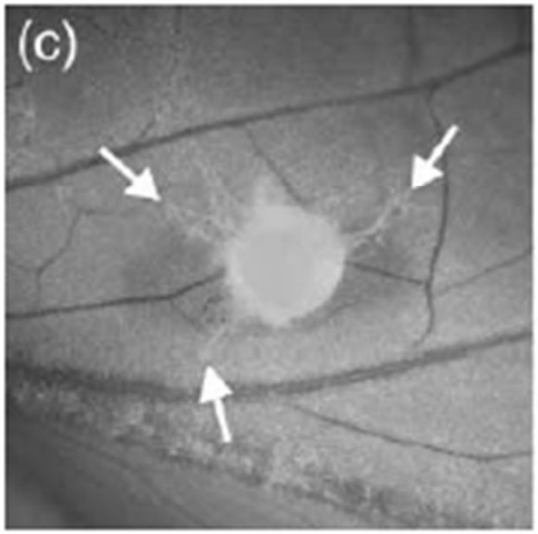
Two new constructs have been created - why?

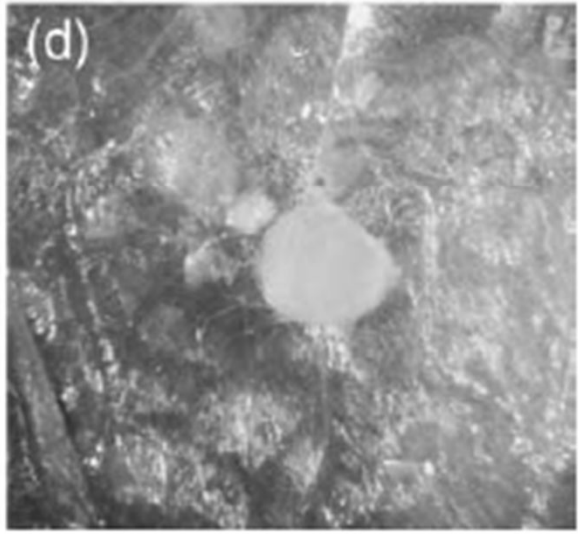
- Problem: - Original wheat *oxox* sequence reported to be glycosylated differently in transgenic plants – affecting performance
- Expression in first lines is still under investigation
- Improved *oxox (*barley*)* cDNA sequence is available from Syngenta
- A *oxdc* sequence reported by Dias et al. (2006)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

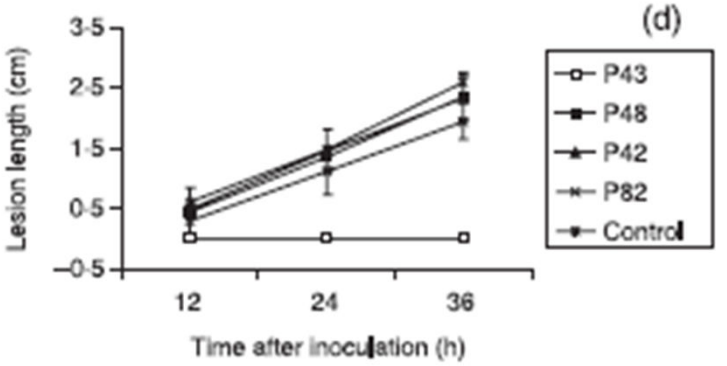
Dias et al 2006. Plant pathology 55:187-193

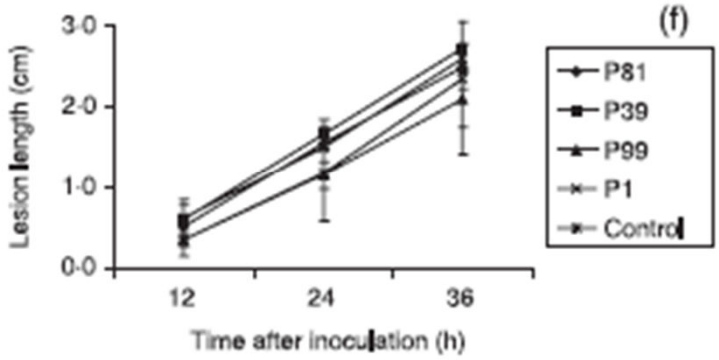


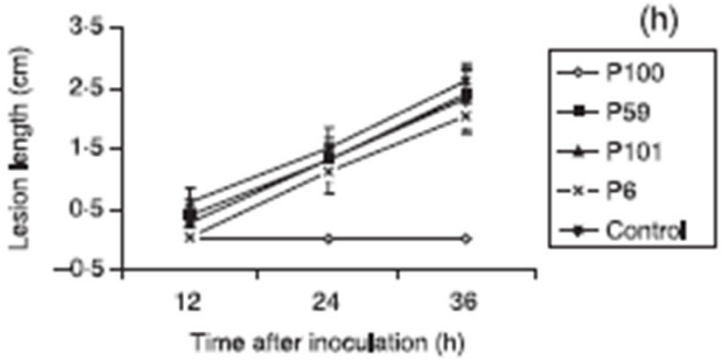












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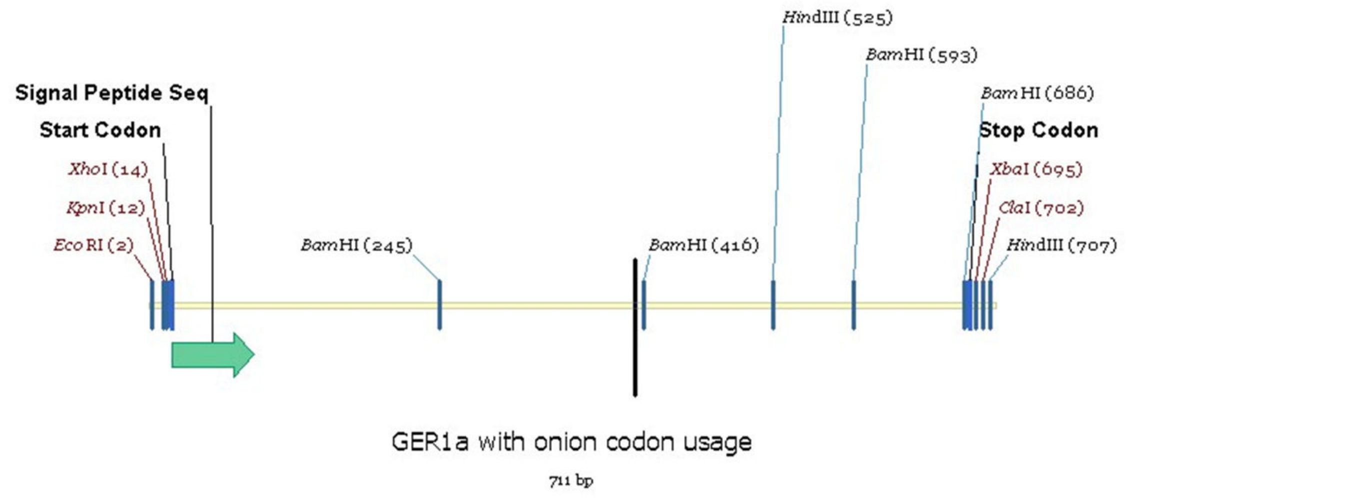
DNA constructs




```
                   KpnI
       EcoRI              XhoI
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      CTTAAGCCAT GGGAGCTCTA CCCTATGAGG TTCTTAAACC CTCGACCTAA CAAATGATAC AACAACAACC GAGGACGATA ATACCGATGA CTAGGACTAG
101   CTTTGCAGGA TTTTTGCGTG GCTGATTTGG ATGGAAAGGC TGTGTCCGTG AATGGACATA CTTGCAAGCC TATGTCCGAA GCTGGAGATG ATTTTTTGTT
      GAAACGTCCT AAAAACGCAC CGACTAAACC TACCTTTCCG ACACAGGCAC TTACCTGTAT GAACGTTCGG ATACAGGCTT CGACCTCTAC TAAAAAACAA
                                                   BamHI
201   TTCCTCCAAG TTGACTAAGG CTGGAAATAC TTCCACTCCT AATGGATCCG CTGTGACTGA ATTGGATGTG GCTGAATGGC CTGGAACTAA TACTTTGGGA
      AAGGAGGTTC AACTGATTCC GACCTTTATG AAGGTGAGGA TTACCTAGGC GACACTGACT TAACCTACAC CGACTTACCG GACCTTGATT ATGAAACCCT
301   GTGTCCATGA ATAGAGTGGA TTTTGCTCCT GGAGGAACTA ATCCTCCTCA TATTCATCCT AGAGCTACTG AAATTGGAAT GGTGATGAAG GGAGAATTGT
      CACAGGTACT TATCTCACCT AAAACGAGGA CCTCCTTGAT TAGGAGGAGT ATAAGTAGGA TCTCGATGAC TTTAACCTTA CCACTACTTC CCTCTTAACA
                     BamHI
401   TGGTGGGAAT TTTGGGATCC TTGGATTCCG GAAATAAGTT GTACTCCAGA GTGGTGAGAG CTGGAGAAAC TTTTGTGATT CCTAGAGGAT TGATGCATTT
      ACCACCCTTA AAACCCTAGG AACCTAAGGC CTTTATTCAA CATGAGGTCT CACCACTCTC GACCTCTTTG AAAACACTAA GGATCTCCTA ACTACGTAAA
                             HindIII                                                                    BamHI
501   TCAGTTTAAT GTGGGAAAGA CTGAAGCTTA CATGGTGGTG TCCTTTAATT CCCAGAATCC TGGAATTGTG TTTGTGCCTT TGACTTTGTT TGGATCCAAT
      AGTCAAATTA CACCCTTTCT GACTTCGAAT GTACCACCAC AGGAAATTAA GGGTCTTAGG ACCTTAACAC AAACACGGAA ACTGAAACAA ACCTAGGTTA
                                                                                                           XbaI
                                                                                             BamHI         ClaI
601   CCTCCTATTC CTACTCCTGT GTTGACTAAG GCTTTGAGAG TGGAAGCTGG AGTGGTGGAA TTGTTGAAGT CCAAGTTTGC TGGAGGATCC TGATCTAGAA
      GGAGGATAAG GATGAGGACA CAACTGATTC CGAAACTCTC ACCTTCGACC TCACCACCTT AACAACTTCA GGTTCAAACG ACCTCCTAGG ACTAGATCTT
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      ClaI
701   TCGATAAGCT T
      AGCTATTCGA A
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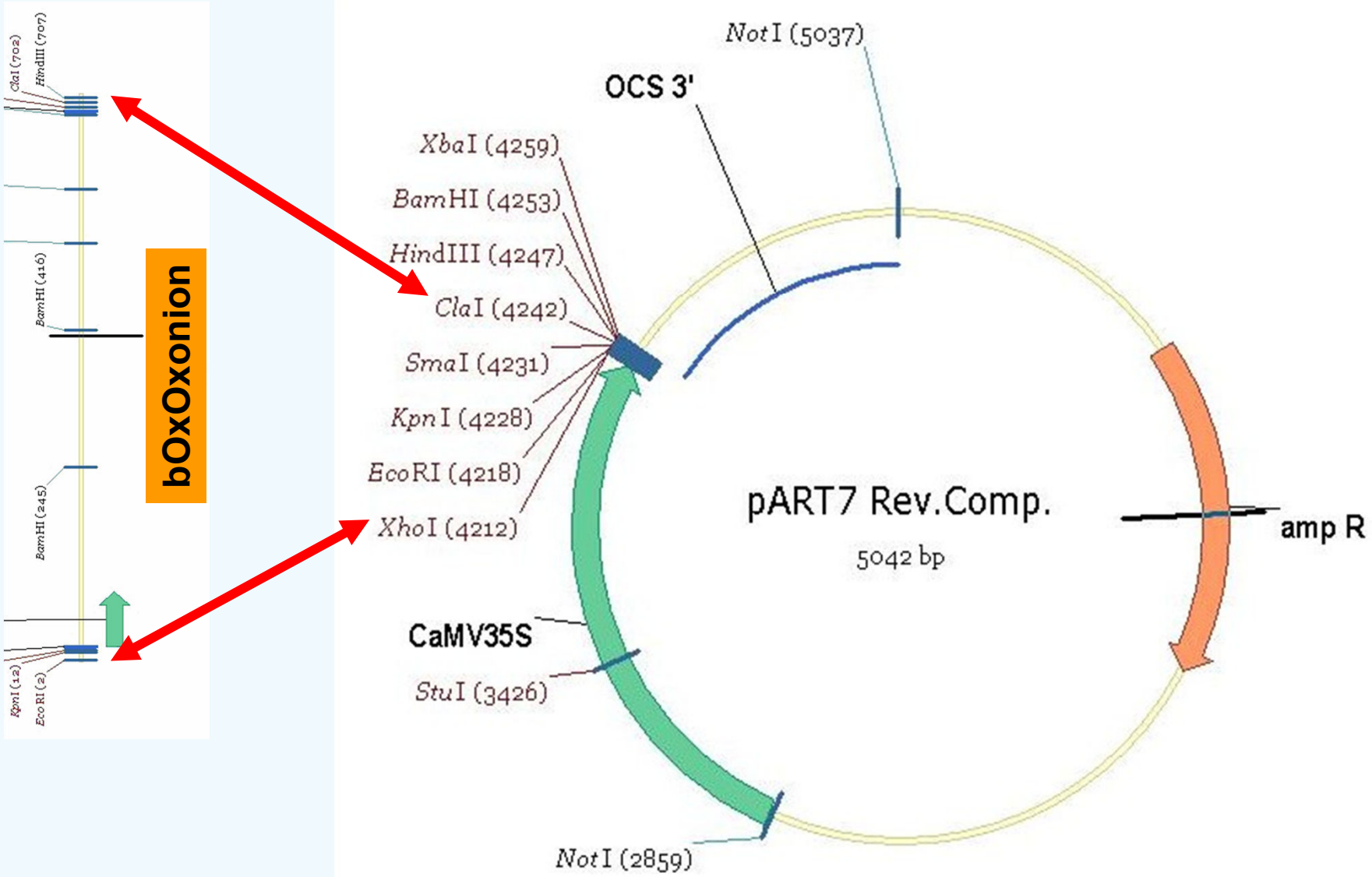
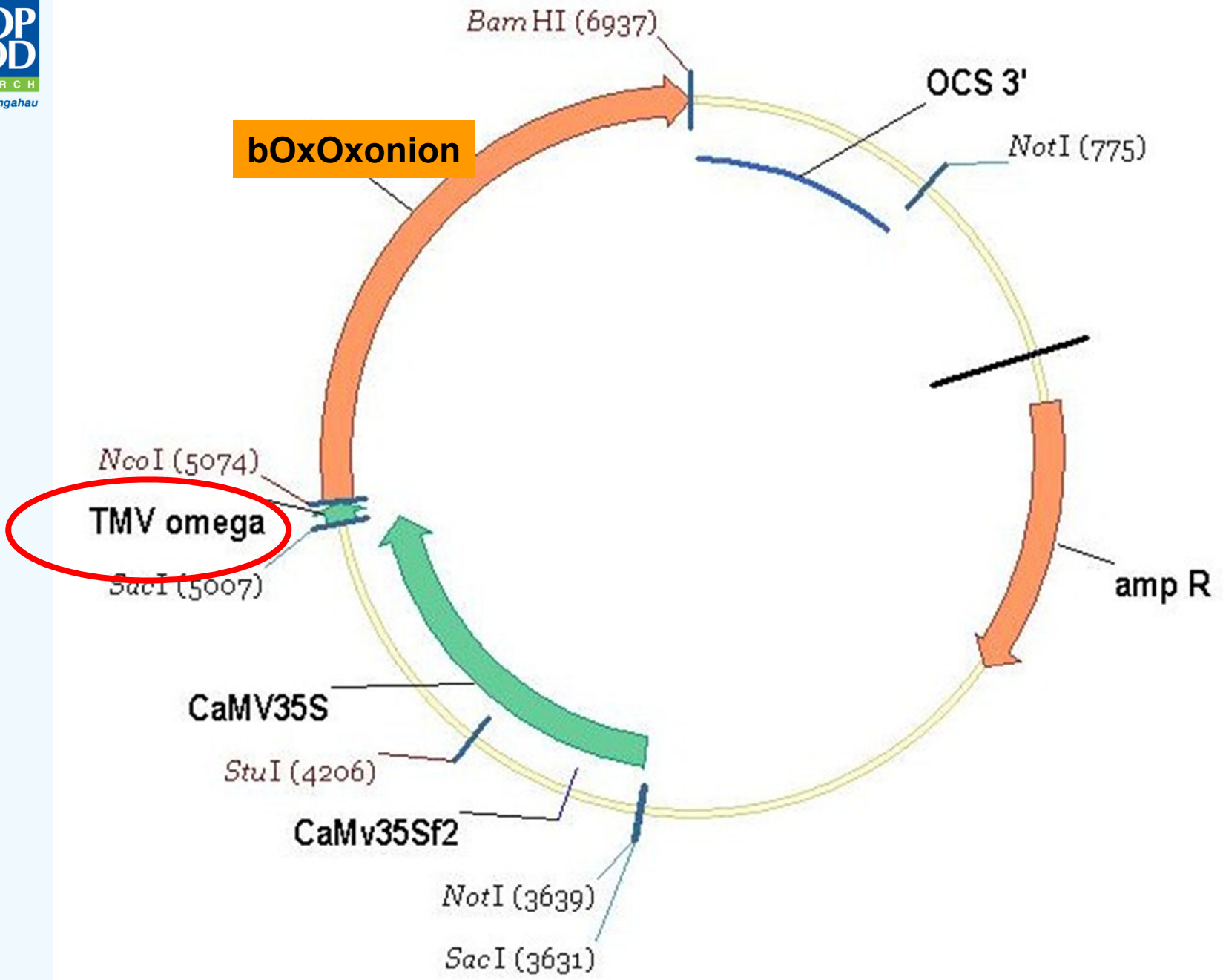
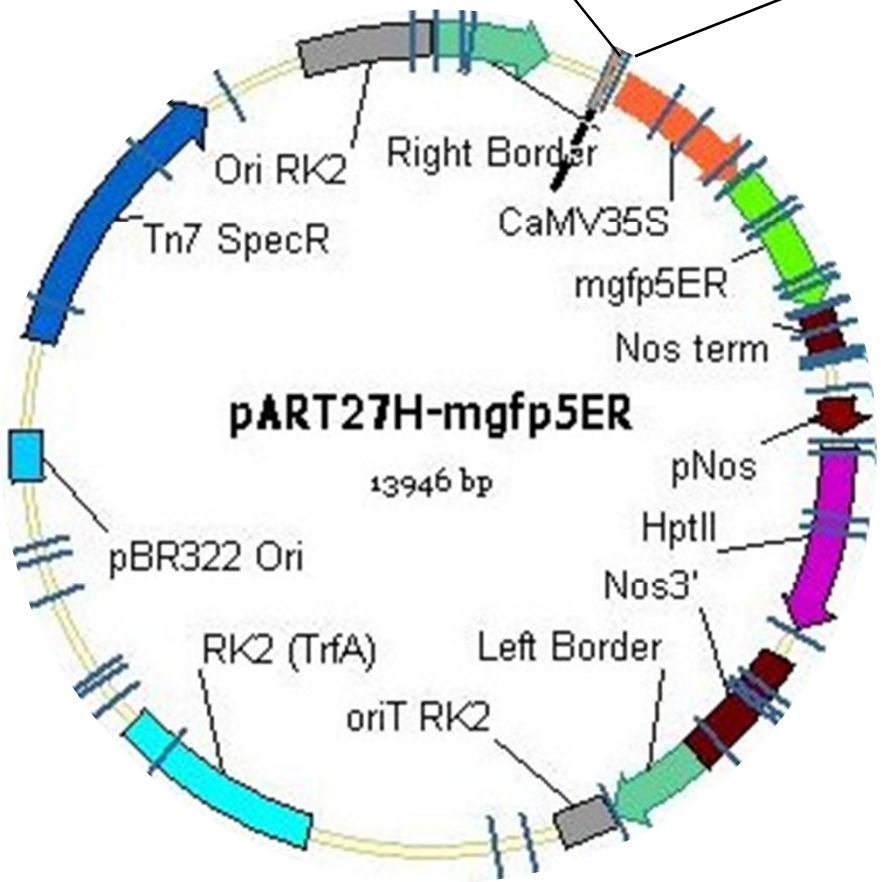

ClaI (702)
HindIII (707)
BamHI (416)
BamHI (245)
KpnI (12)
EcoRI (2)
bOxOxonion
NotI (5037)
OCS 3'
XbaI (4259)
BamHI (4253)
HindIII (4247)
ClaI (4242)
SmaI (4231)
KpnI (4228)
EcoRI (4218)
XhoI (4212)
pART7 Rev.Comp.
5042 bp
amp R
CaMV35S
StuI (3426)
NotI (2859)


BamHI (6937)
OCS 3'
bOxOxonion
NotI (775)
NcoI (5074)
TMV omega
SacI (5007)
amp R
CaMV35S
StuI (4206)
CaMv35Sf2
NotI (3639)
SacI (3631)

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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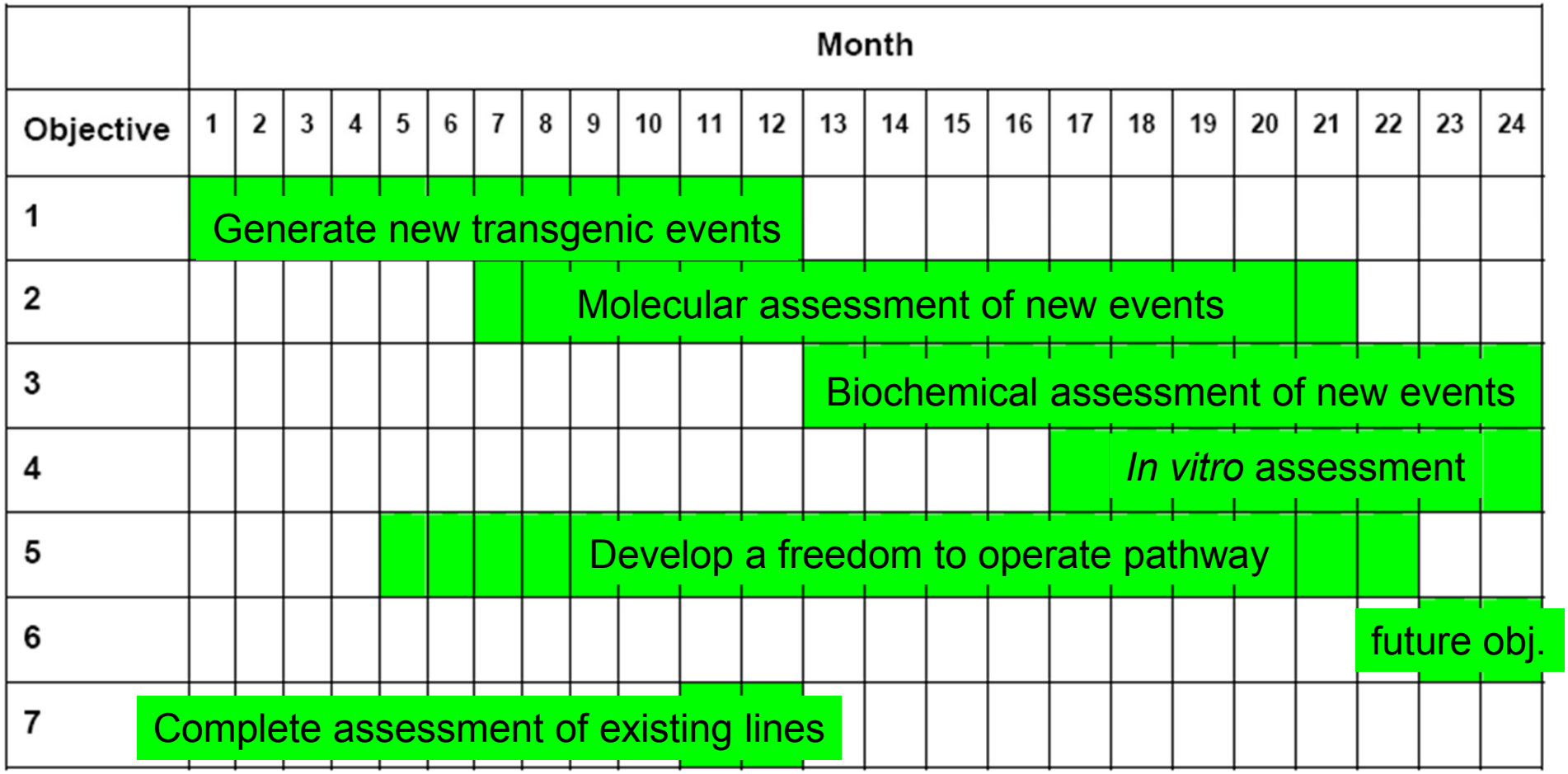
Private Bag 4704, Christchurch, New Zealand

KNOWLEDGE AND VALUE FROM SCIENTIFIC DISCOVERY

Future research 2008/9

Timeline



Acknowledgements