

Plant & Food
RESEARCH

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Biotech approaches to AWR – perils and pitfalls

Proposal prepared by:
Colin Eady February 2012

Allium biotechnology at The New Zealand Institute for Plant & Food Research Limited

- Allium Biotechnology holds great promise:
 - Puts us in a unique position to study:
 1. Intractable problems
 2. Interesting allium specific traits
- Perils and pitfalls of biotech research
- State of play for AWR research

Examples of intractable problems

- Weed control



- Virus resistance?



- AWR resistance ?



Examples of gene function studies

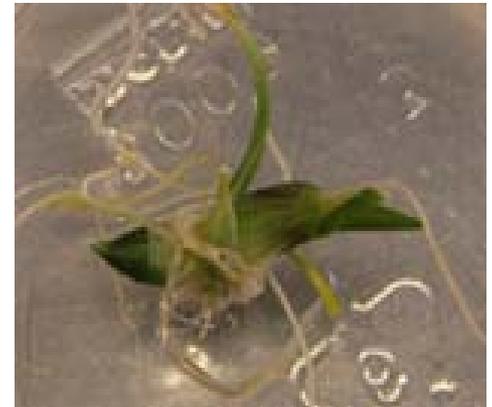
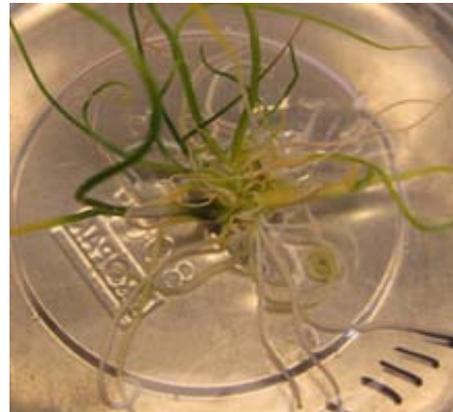
Tearless onion



regulators of colour



regulators of bulbing



Perils and pitfalls

Sometimes we have to learn from our own mistakes



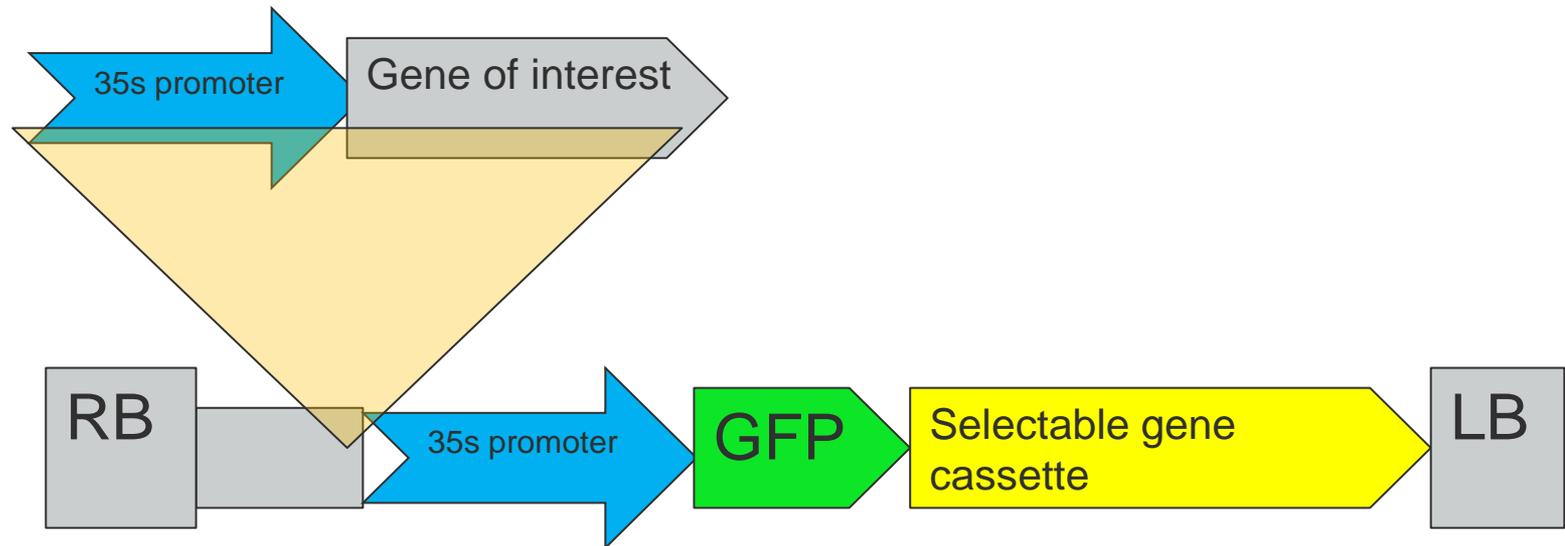
Compliance, regulatory issues, etc. err on the side of caution



"WELL MASTER JACK! WE HAVE REASON TO BELIEVE YOU'RE GROWING A G.M. CROP WITHOUT A LICENCE."

Pitfall

2 separate 35s promoters cause interference and do not function correctly



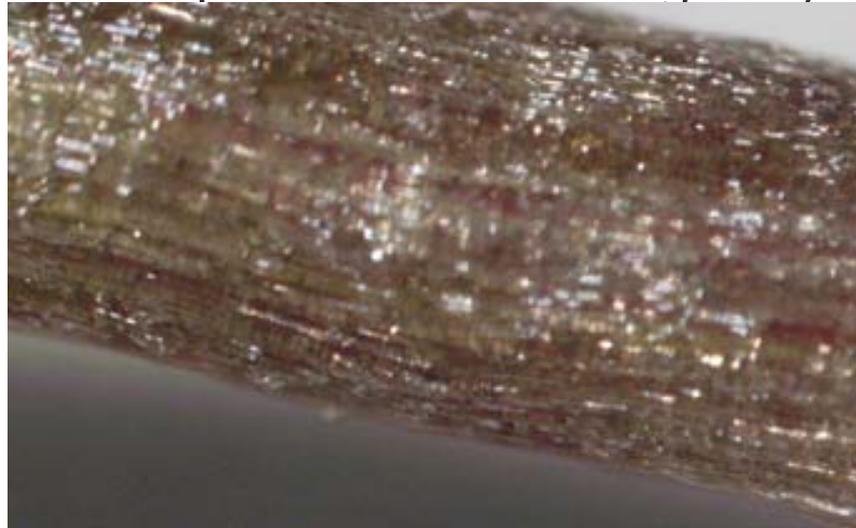
One 35s promoter



Two 35s promoters



We have found that in allium this expression problem is very common. This has complicated our results greatly



Peril

- **Compliance / legal / political problems**
 1. Unrelated? Related compliance problems
 2. Collateral damage / Collaborator problems
 3. NZ political appetite for biotech
 4. Company response to 1 & 3 above

Unrelated? Compliance issue #1 GM Brassica Field Trial

2007



2008–09





MAF Dodging Plant & Food's \$10m GE Liability

Monday, 29 June 2009, 9:29 am
Press Release: Soil and Health Association

Soil & Health Association of New Zealand
Est. 1941) Publishers of ORGANIC NZ

28 June 2009

MAF Wet Bus Ticket Response is Dodging Plant & Food's \$10million GE Liability.

The final response of MAF's biosecurity and enforcement teams following major breaches of consent by Plant & Food Research (P&F) at its GE brassica (broccoli, cauliflower, cabbages etc.) field trial during 2008, is a massive insult to the more than 930 submitters who opposed the field trial, and shows the corruption of government, according to the Soil & Health Association of NZ

Unrelated compliance issue #2 GM Arabidopsis 2009

GE weeds spread from containment glasshouse at Lincoln | NATIONAL News

Page 1 of 1

GE weeds spread from containment glasshouse

Published: 8:31PM Monday November 30, 2009 Source: NZPA



Statutory liability under HSNO Act

HSNO Act General liability and enforcement

Failing to comply with any controls imposed by any approval granted under the HSNO Act – Section 109 (1)(e)(i)

(1) Every person commits an offense under this act who-

(e) : Fails to comply with (i) any controls imposed by an approval granted under this act.

caused a restricted organisms to leave a containment facility – Section 154(m)

Civil liability (New Organisms only) – acts and omissions while in breach of the HSNO Act

- Pecuniary penalty orders, plus civil liability for loss or damage

Up to \$10 million or 10% of company turnover, which ever is greater

Collateral damage, contractual stalemate, freedom to operate

Bloomberg

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Bayer Agrees to Pay \$750 Million to End Lawsuits Over Gene-Modified Rice

By Andrew Harris and David Beasley - Jul 2, 2011 4:01 PM GMT+1200

ADD TO QUEUE

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A [Bayer AG \(BAYN\)](#) unit agreed to a \$750 million settlement resolving claims with about 11,000 U.S. farmers who said a strain of the company's genetically modified rice tainted crops and ruined their export value.

Consequence

- 1000 GM onion and garlic plants, 5 years work, destroyed
- Two commercial collaborations lost
- GM glasshouse and field site closed
- Material culled and moved to sub optimal Biotron at the University



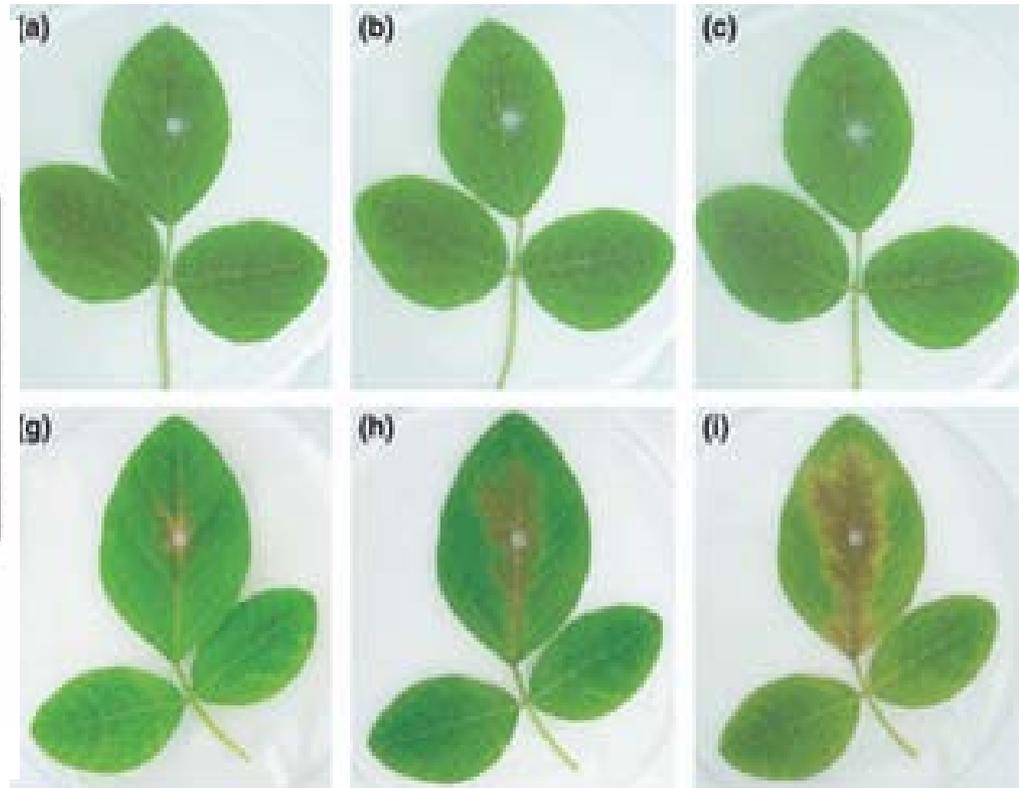
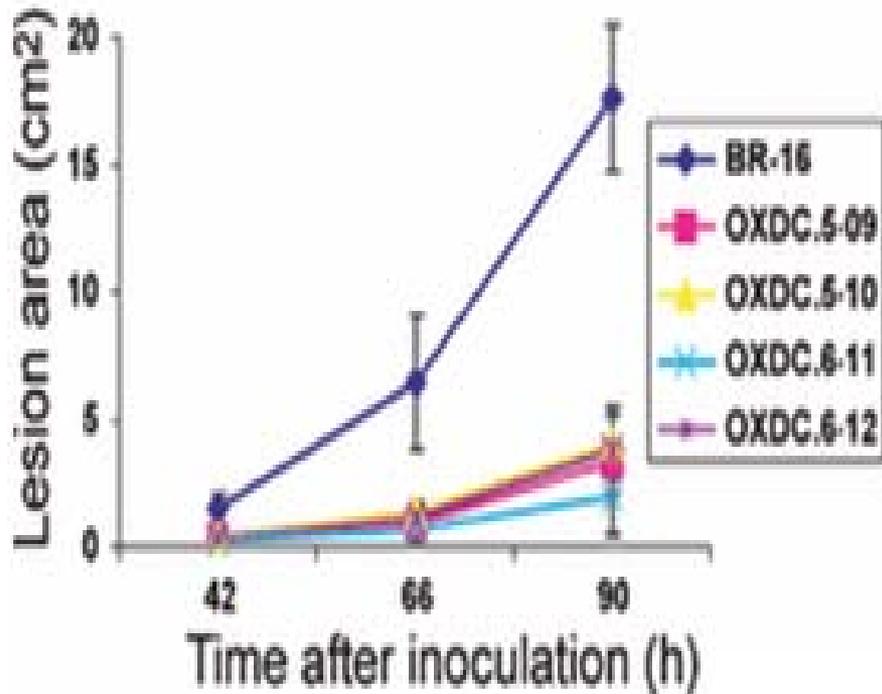
GORAB – Biotech approach to AWR – progress

Importantly the hypothesis is still valid

High resistance to *Sclerotinia sclerotiorum* in transgenic soybean plants transformed to express an oxalate decarboxylase gene

W. G. Cunha et al.

Plant Pathology 2010



Summary at end of 2010

- Our oxalate decarboxylase construct is difficult to transform into plants and expression is often poor

Future work

1. Continue assessment of current and future *oxdc* Alliums
2. Develop refined vacuolar-targetted *oxdc* construct
3. Produce a white rot resistant allium for assessment
4. Develop a business model that will deliver these benefits to the industry

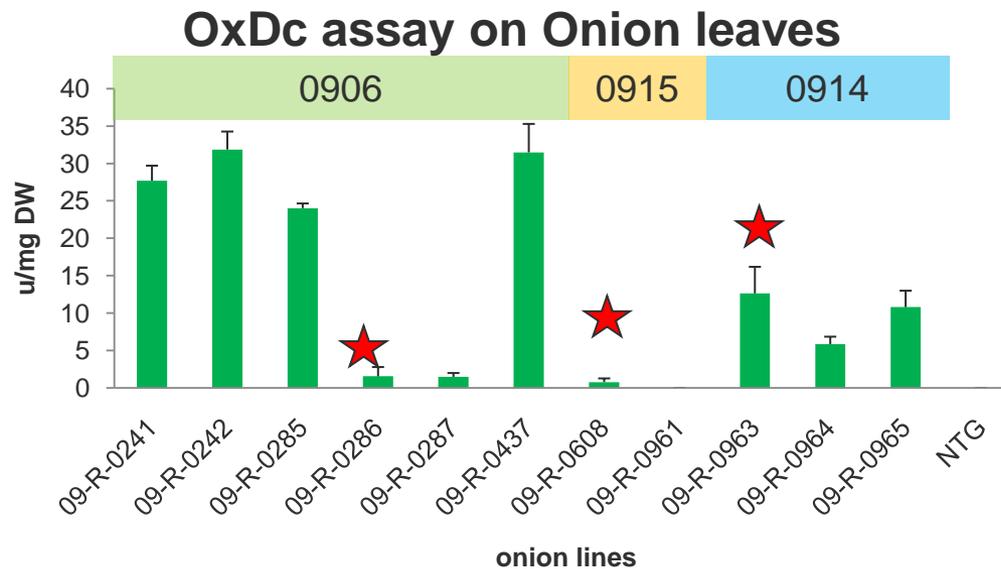
1. Continue assessment of current and future *oxdc* Alliums

GFP and OxDc very variable

GFP



OxDc



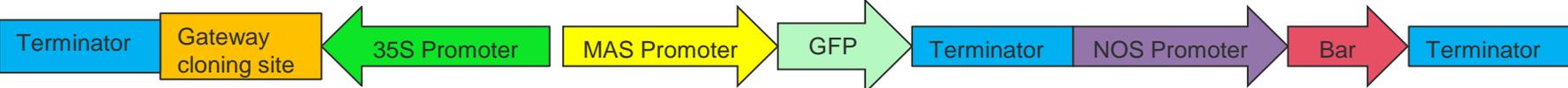
2. Develop refined vacuolar-targetted *oxdc* construct.

– Back to the drawing board

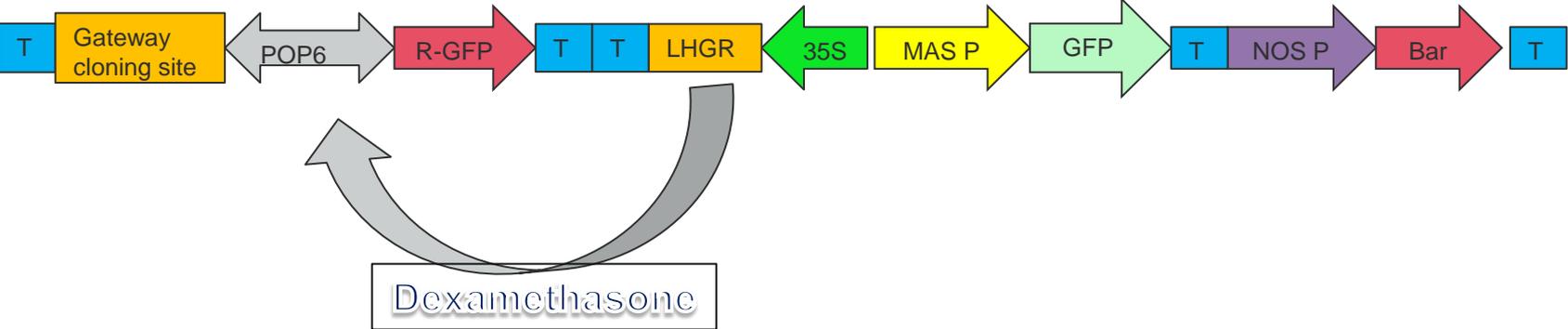
1. Need new transformation constructs to try and avoid the transcriptional gene silencing problems
2. Need to test the targeting of OxDc to different tissues
 - » Early work demonstrated that vacuolar targeting conferred better resistance
 - » Also discovered that first part of the original *oxdc* gene is potentially a fungal secretory sequence
3. Return to model tobacco system for proof of concept research

New transformation constructs

1. Overexpression



2. Inducible



New targeted expression of OxDC

Two issues to test :

- Original OxDc had a fungal secretory sequence – is this a problem
- Evidence that targetting OxDc to vacuole may function more efficiently

New OxDc genes

- Modified OxDc (minus the fungal secretory sequence)
- Vacuole targetted OxDc

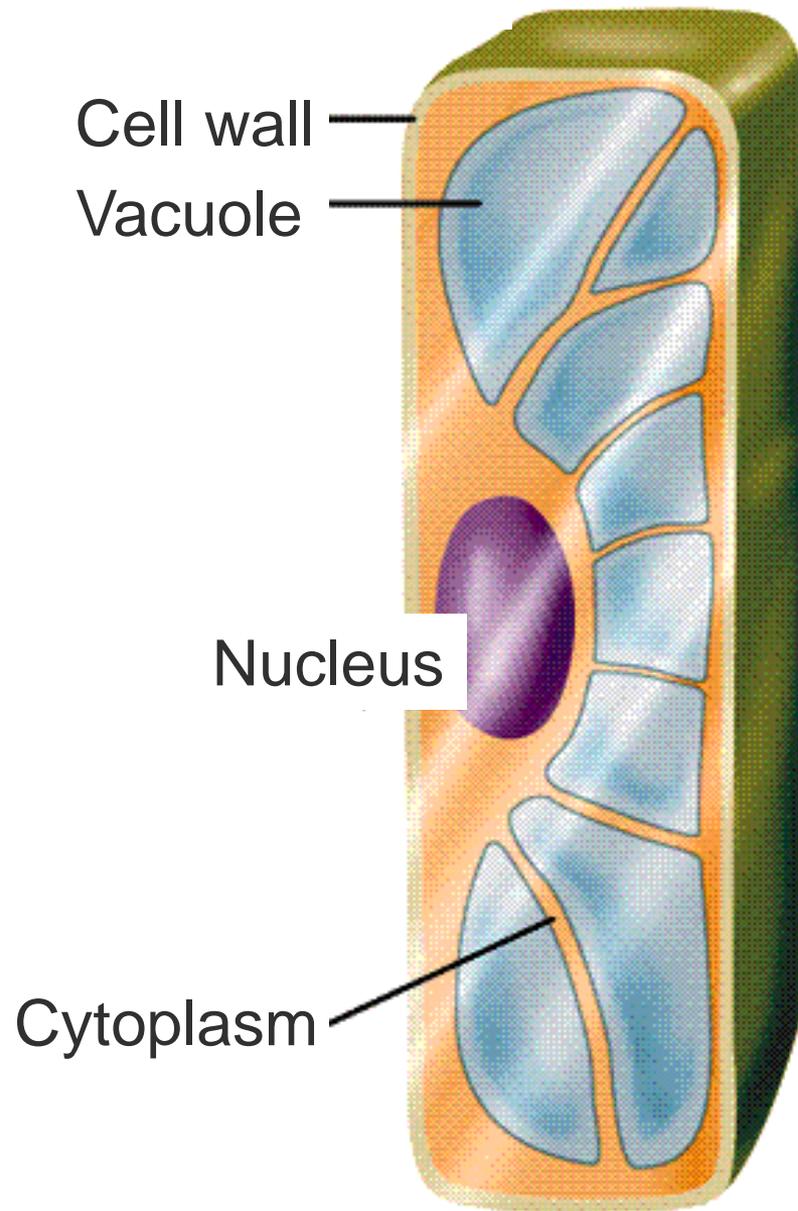
Expression check

Modified OxDC/GFP fusion

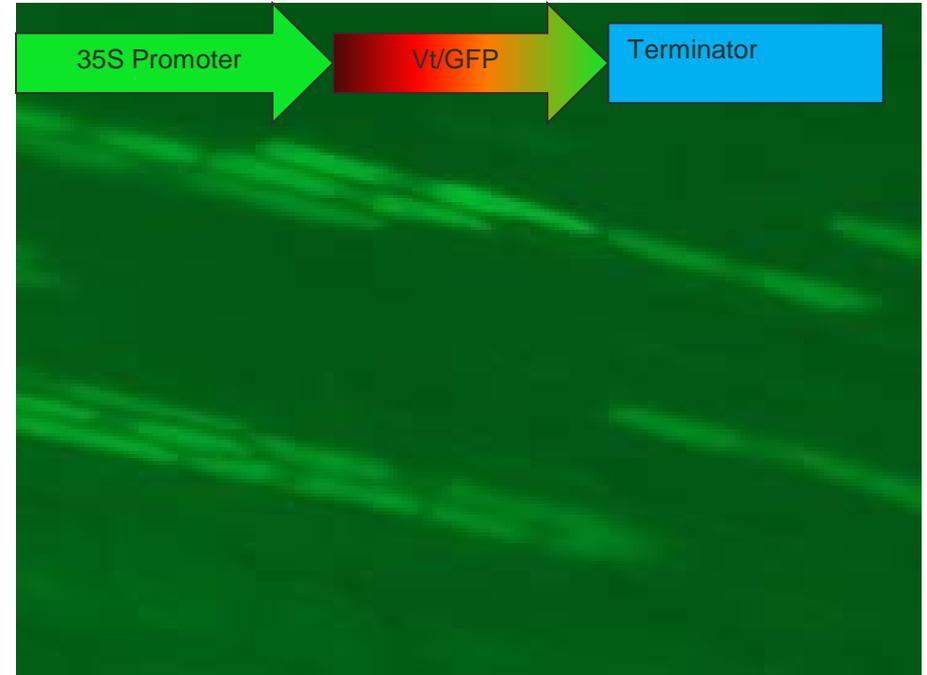
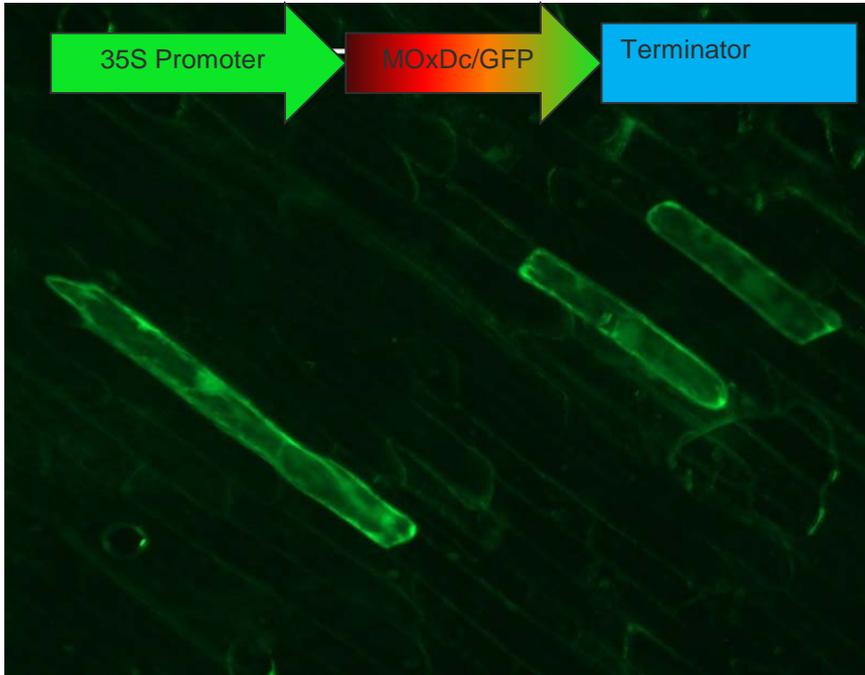


Vt/GFP





Expression check for MOxDc and Vt sequence



Summary, current and future work

Summary

- Hypothesis is still valid but technical and 'compliance' issues have put project behind

Current work (next 4 months)

- Regenerate transgenic tobacco containing the new constructs

Future

- Assess oxalate decarboxylase activity
- Assess activity against white rot
- Select best construct and transform garlic

Acknowledgements

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