The potential for pest control in crop plants using gene silencing technology

Michael G K Jones
WA State Agricultural Biotechnology Centre, Murdoch University, Perth, Western Australia
Overview

• What is gene silencing (RNAi) technology?
• Focus on pest control – nematodes and aphids
• Why are nematodes that attack plants important?
• Host-pathogen interactions
• ‘Host Induced Gene Silencing’ (HIGS)
• Why are aphids that attack plants important?
• Broader applications of gene silencing in plant biotechnology
RNAi in plant nematodes

Mechanism of RNAi in nematodes

Bakhetia et al (2005)
Nematodes – hidden pests of crop plants

- Damage roots and predispose them to secondary infections
- Poor water use efficiency – prone to wilting
- Poor nutrient use efficiency – yellowing
- Reduced yields and quality
- Premature senescence and plant death
The problem

Young corn crop damaged by needle nematode

Beet cyst nematode damage L, nematicide treated R

Barley crop infested with Meloidogyne naasi

Damage to soybean by soybean cyst nematode

Young corn crop damaged by needle nematode
Main nematode pests

• Root knot nematodes
• Cyst nematodes
• Root lesion nematodes
Major losses caused by root lesion nematodes on wheat in Australia

- Root lesion nematodes - southern and western grain belt, cost the wheat industry in this region alone at least A$190 million pa in lost production (Vanstone et al, 2008).
- *Pratylenchus thornei*
- *P. penetrans*
- *P. neglectus*
- *P. teres*
- *Radopholus nativus*
Root lesion nematodes (RLNs, *Pratylenchus* spp.)

- Third most economically important genera of PPNs
- Over 60 species described (*P. penetrans, P. thornei, P. neglectus, P. zeae, P. vulnus* and *P. coffeae*).
- Wide host range – many crops attacked
- All life stages are wormlike and mobile
- Cause extensive necrosis (lesions) by their feeding and migration
- Damage is aggravated by secondary infection with soil pathogens such as fungi and bacteria.
Root lesion nematode damage to wheat and maize

7 to 15% reduction in wheat yield in Western Australia

corn, USA
Maintaining defined RLN populations

Maintenance of *Pratylenchus* cultures isolated from wheat on carrot pieces

**Time course of RLN (Pt) multiplication on carrot mini-discs**

- Purple bars: Number of nematodes per disc
- Yellow bars: 50 nems

**Mini carrot discs to assess effects of treatments**
Search for target genes

Sequenced the transcriptomes of *Pratylenchus* spp and *H. avenae*

- Mixed stages of *P. thornei* adults: juveniles: eggs, (ratio 3:2:1)
- Total RNA extracted
- Processed for Roche 454 GS FLX transcriptome analysis
- Reads assembled and annotated

Types of targets for control

• Effectors – usually secreted from gland cells
  – Enable pests to migrate through plant tissues
  – Suppress or evade host defences
  – Required to develop long term feeding site (giant cells, syncytia)

• Genes vital for the nematode life cycle
  – Generic
Identification of candidate genes for RNAi to control RLNS

- **P. coffeae** (Haegeman et al. 2011)
- **P. zeae** (Submitted)
- **P. thornei** (Nicol et al. 2012)

ESTs and genomic sequences from other nematode species

- Cell wall degradation
- Proteases
- Suppression of host defences
- Known/unknown function

>30 genes identified and characterised

In vitro RNAi In planta
Conditions to make root lesion nematodes take up dsRNA have been optimised

- Fluorescein isothiocyanate (FITC)
- Neurostimulant
- Spermidine derivatives

Analysis of RNAi in RLNs after soaking

- Soaking in optimised medium containing dsRNA
- RNA extraction
- Quantitative PCR for target genes expression
- Microscopy for phenotypic effects
- Mini carrot disc infection
- Count nematodes to determine effects of soaking
- 5 weeks
Examples of target genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>pat-10</th>
<th>unc-87</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function</td>
<td>Regulates body wall muscle contraction</td>
<td>Maintain body wall muscle</td>
</tr>
<tr>
<td>RNAi phenotypes in C. elegans</td>
<td>Paralysis (Rual et al. 2004)</td>
<td>Severe paralysis in larvae and limp paralysis in the adult (Simmer et al. 2003)</td>
</tr>
</tbody>
</table>
Treatment with dsRNA of *pat-10* and *unc-87* causes abnormal phenotypes

- **dsRNA - Ptunc-87**: Loss of sense of direction - *P. zeae*
- **dsRNA - Pzpat-10**: ‘Head banging’ - *P. thornei*

Paralysis - *P. thornei*  Wavy movements - *P. zeae*
Substantial reduction in target gene expression *(P. thornei* more susceptible to RNAi than *P. zeae)*

![Graph showing fold reduction for P. thornei and P. zeae with dsRNA treatments.]

**Graph:**
- **Y-axis:** Fold reduction
- **X-axis:** Types of dsRNA treatments
  - Pzpat-10 dsRNA
  - Ptunc-87 dsRNA

Legend:
- **Blue bars:** P. thornei
- **Red bars:** P. zeae
Silencing of *pat-10* and *unc-87* significantly reduces reproduction of *P. thornei* on carrot discs

- *P. thornei* incubated with dsRNA, washed, **cultured for 5 weeks** on mini carrot discs.
- Reproduction of *P. thornei* was significantly reduced (**up to 81% reduction**, p<0.05) after treatment with dsRNA of *Pzpat 10* or *Ptunc87*
Principle of transgenic resistance (HIGS)

- *In planta* delivery of dsRNA to nematode

  Nematode ingests siRNAs which silence vital genes: nematodes unable to grow/reproduce

  Make transgenic plants expressing dsRNA to target gene in nematode

Bakhetia et al (2005)
RNAi constructs to silence nematode

Hairpin dsRNA
Application of RNAi to control RLNs in wheat

- immature embryos dissected and cultured
- particle bombardment with constructs
- cultured embryos under selection
- shoot regeneration
- transgenic plants
GM wheat challenge with *P. thornei*
Extracting nematodes
Strong reduction in reproduction of RLNs in transgenic wheat plants

Total nematodes (roots + soil)
Transgenic resistance to beet cyst nematode in Arabidopsis
RNAi for aphid control

- Green Peach Aphid (GPA): *Myzus persicae*
- Polyphagous (>40 families)
- Feeding damage + vector (>100 viruses) CMV, BYV, BWYV, BMV, LMV, etc.
- Losses worth millions of $
Approach

- Genomic analysis, bioinformatics
- Identifying target genes
- Clone, check
- Generate dsRNA to target genes
- Feed aphids dsRNA
- Assess effects
- Results promising
Feeding on artificial diets plus dsRNA for some targets kills aphids

Reduction in gene expression 24 hr after feeding

Fold reduction of gene expression

30% sucrose (no dsRNA)  GFP dsRNA  Target dsRNA

Treatments

Fed with dsRNA

Control
• What sequences generate small RNAs?
• How can we increase the percentage down-regulation of target genes?
• Is it possible to prevent pre-processing of long dsRNAs by plants?
Full crop protection from an insect pests by expression of long dsRNAs in plastids

- Plants pre-process long dsRNA
- Long dsRNAs can be generated in chloroplasts
- dsRNA accumulates in plastids
- Chloroplast transformation by particle bombardment and selection
- dsRNA targeting β-actin of Colorado beetle expressed in potato chloroplasts were lethal to larvae (100% mortality)

Public acceptance of RNAi?

• Reduce possibility of off-target effects – eg bioinformatic searches, reduce size of RNA sequence used, employ artificial microRNA vector

• Only a fragment of RNA/gene used, this does not involve production of a protein, so expect better acceptance by regulators

• Some publications suggesting small RNA can enter the blood stream of humans have been refuted
Other applications of RNAi

• Modifying flower colour – ‘Moon’ carnations
• Resistance to corn rootworm (Monsanto)
• Reducing browning ‘Artic Apples’ Okanagan Speciality Fruits (RNAi to PPO in rootstock)
• Healthier soybean oil
• Bowel health (CSIRO) – resistant starch
The blurring of GM and non-GM technologies

Most wheat contains rye genes introduced artificially!

Modified from J Dunwell
BioDirect™ Technology

- Biodirect technology is a non-transgenic alternative mode of RNAi
- Herbicide, insect and virus control by topical application of small RNAs for crop protection and other products

Conclusions

• RNAi is a powerful technology with many applications in agriculture
• Broad spectrum resistance to root lesion nematodes, conferring 7-15% yield increase, and potential to confer resistance to a number of nematode species at the same time
• Applications include transgenic crop resistance to insect pests and other diseases, improved properties for human health
• Expect better acceptance of RNAi technology than for transgenics that generate an encoded protein product
• Non-transgenic applications – spray on applications to control pests and diseases
Nemgenix Pty Ltd

WA State Agricultural Biotechnology Centre, Perth

AGRICULTURAL BIOLOGICALS - COLLABORATION OPPORTUNITIES

(NON-CONFIDENTIAL)
Acknowledgements

• Plant Biotechnology Research Group
• Australian Research Council
• Australia India Strategic Research Fund
• Commercialisation Australia
• Nemgenix Pty Ltd