

New gene disruption technology to understand blackleg disease

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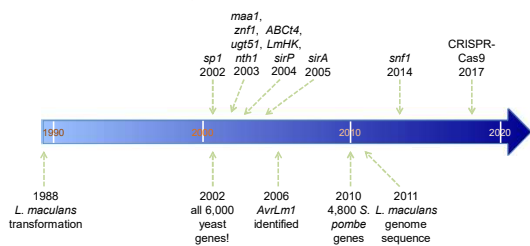
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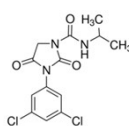
UM00050 “ ‘Proof of concept’ for approaches designed at increasing disease resistance in pathogens of canola ”



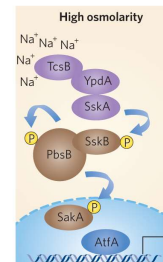
L. maculans genes are difficult to manipulate



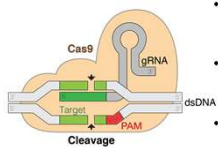
Iprodione resistance emerges at high frequency



- Iprodione (a dicarboximide fungicide) was used from ~1996-2001 on blackleg.
- No longer registered for blackleg disease.
- Resistant strains were isolated by Fran Lopez-Ruiz (Curtin University).
- Resistance is due to mutations in the gene encoding the HOS1 histidine kinase in the HOG pathway.



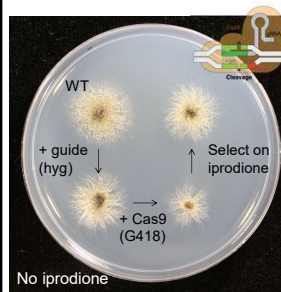
Positive selection as a method to develop CRISPR-Cas9



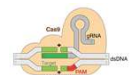
- Cas9 endonuclease expressed in *L. maculans* using a constitutive (actin) promoter.
- Guide RNA driven by a *L. maculans* promoter.
- Two rounds of transformation used to introduce each construct into the genome of *L. maculans*.
- For more information, see Idnurm et al. Dec. 2017 *Fungal Biology & Biotechnology*

Image: wikipedia

Positive selection as a method to develop CRISPR-Cas9



Cas9-induced mutations in *hos1*



Strain	Avr1 sequence	Cleavage
W114 1998	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
A10471	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
A10448	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
A10475	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
A10469	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
A10477	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
ZC6	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
A10478	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
TC2	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	
TC3	ACTTGGGGCAGGCTACAGTACAGTTCCTGAGGGTACTGGAAAACCTTCAGCGAAACCTGAAAGTGTATGGCCATGAACTTCAGAACTCAGTTCGAGAAATTCGAGAAATC	

- All iprodione-resistant strains have mutations in the *hos1* gene, near the guide RNA.

Other genes can be mutated with CRISPR/Cas9

- Genes encoding avirulence proteins.
- Genes expressed during stem cankering.
- Cell wall synthesis (or associated) proteins.

Strains with deletions in *AvrLm* genes

- Genes encoding avirulence proteins.
 - No Avr gene confirmed by gene disruption.
 - Complex interactions now known for Avr/R in blackleg.
 - Why are the wild type copies maintained – identify conditions that select for the avirulence copy of the gene.
 - Mutated *AvrLm1* and *AvrLm4*.

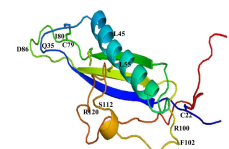
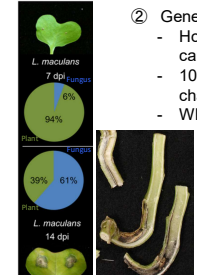

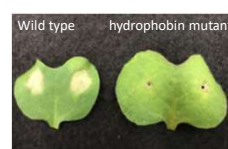


Image: Blondeu et al. 2015

Genes contributing to stem cankering

- Genes expressed during stem cankering.
 - How similar is disease development during stem canker vs. on cotyledons/leaves.
 - 10 genes highly expressed in stems mutated – no change in disease.
 - What proportion of genes for cotyledon disease?

Cell wall synthesis

- Cell wall synthesis (or cell wall associated) proteins.
 - Antifungal drug target (echinocandins target glucan synthesis).
 - Expertise at UoM for analysis of cell wall composition and nucleotide sugar transporters (NSTs).
 - essential for cell wall synthesis and glycosylation of other cell-wall material.

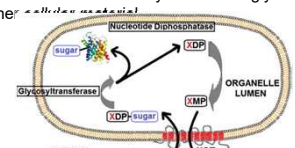
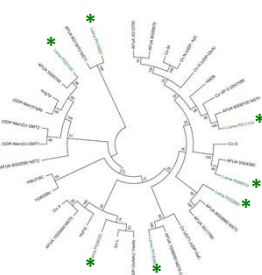
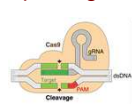
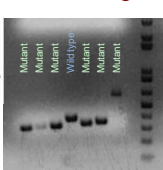
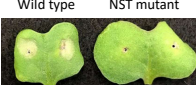


Image: Hedley et al. 2014

L. maculans encodes up to eight NST homologs

Summary and Acknowledgments

- CRISPR/Cas9 is a new and efficient method for gene mutagenesis in *L. maculans*.
- Loss of cell wall components, such as by mutation of genes encoding NSTs, may impact pathogenicity.
 - Using *L. maculans* as a model for fungal disease.
- Thanks to Andrew Urquhart and Naima Tasnim

