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THE UWA INSTITUTE OF AGRICULTURE, & AAFC CANADA

NICK LARKAN, WALLACE COWLING, ET. AL.

The cloning of the first *Brassica napus* resistance gene *LepR3* or "the 10-year mystery of the 2 R-genes in Surpass 400"

ACHIEVE INTERNATIONAL EXCELLENCE

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**2001: Single major dominant R gene identified in Surpass 400** (Li and Cowling 2003)

**Field study with PhD student Caixia Li**

- Measured disease at seedling and adult stage in the field
- Experiment "salted" with stubble of canola varieties collected in Mt Barker, WA in April 2001
- Surpass 400 x Westar genetic study with parents, F1 and F2 population
- Photo: F1 = R (L) Westar = S (R)

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**Li and Cowling (2003) Plant Breeding 122:485 "Single major dominant R gene identified in Surpass 400..."**

**Single dominant gene**

- F1 resistant
- F2 segregates 3:1 R:S
- Similar result in seedling (L) and adult (R) plants
- 91.1% matching reactions (R-R and S-S) between seedling and adult plants
- High variation in the "S" category in the F2, showing segregation of polygenic R?

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**Nick Larkan - postdoc UWA, then AAFC Canada**

**2003 – 2006: UWA - fine mapping of LepR3 gene (ARC LP0210571)**

- At UWA, prepared genetic material and field resistance studies
- At AAFC, comparative mapping with Arabidopsis, and laboratory disease assessment of LepR3 to assess its position on the chromosome.

**2006 – present: AAFC – cloning and avirulence gene determination**

- At AAFC, revised fine mapping, and map-based cloning of LepR3
- 2013: publication New Phytologist (2013) 197: 595–605

**The *Brassica napus* blackleg resistance gene *LepR3* encodes a receptor-like protein triggered by the *Leptosphaeria maculans* effector AVRLM1**

N. J. Larkan<sup>1,2</sup>, D. J. Lydiate<sup>1</sup>, L. A. P. Parkin<sup>1</sup>, M. N. Nelson<sup>2</sup>, D. J. Epp<sup>1</sup>, W. A. Cowling<sup>2</sup>, S. R. Rimmer<sup>1</sup> and M. H. Borhan<sup>1</sup>

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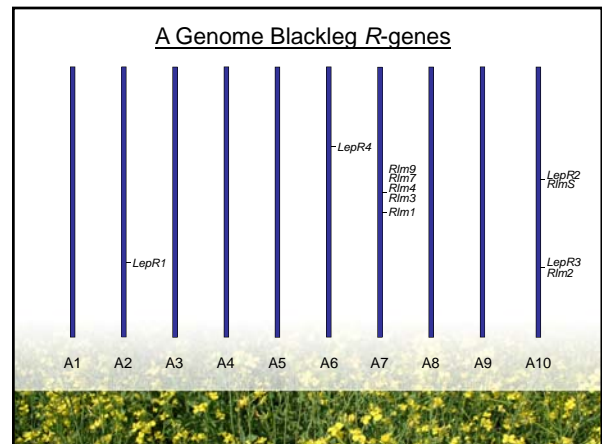
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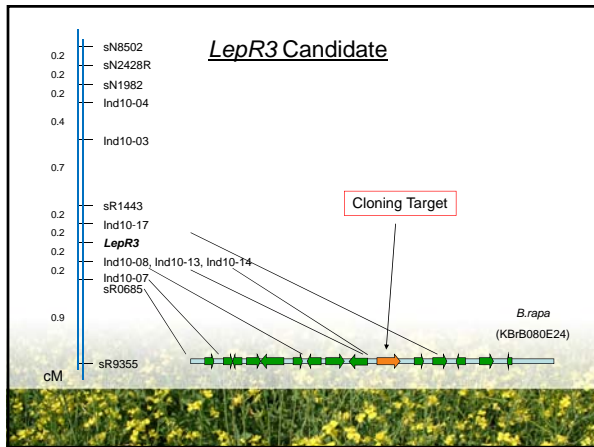
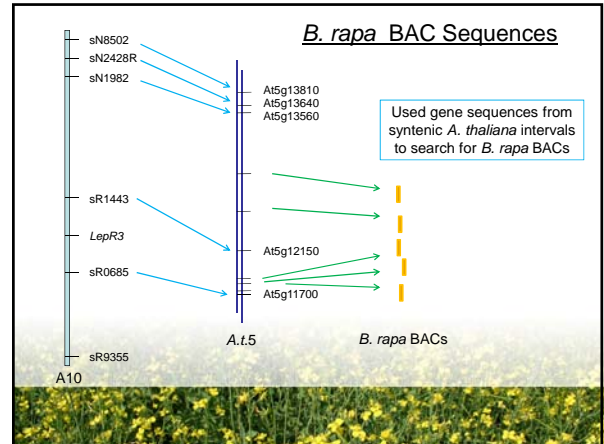
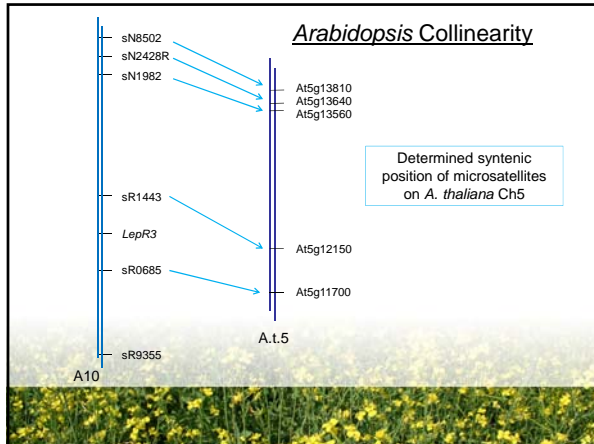
**Conclusions of cloning work**

**Surpass 400 contains an "R" gene named *LepR3* on chromosome 10**

- Unusually, this R gene interacts with *AvrLm1* to invoke resistance reaction
- AvrLm1* also interacts specifically to invoke resistance with *Rlm1* (Columbus) on chromosome 7
- Rlm1* and *LepR3* cannot currently be distinguished by differential phenotypic reactions
- This demonstrates redundancy in resistance genes: one avirulence protein interacts with two specific R-proteins
- Unfortunately for Australia, isolates with *avrLm1* were prevalent before the introduction of Surpass 400, therefore resistance was quickly "broken" by selection for this strain.

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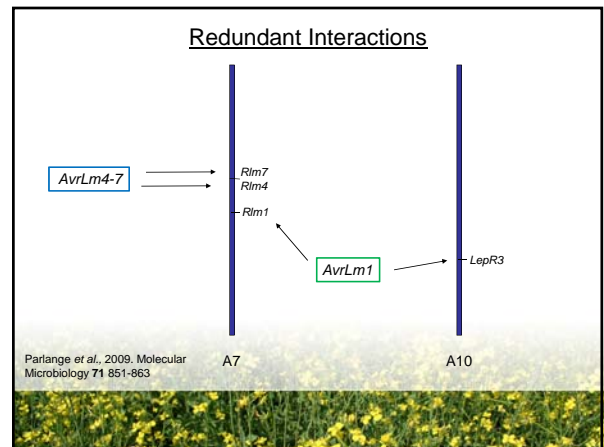
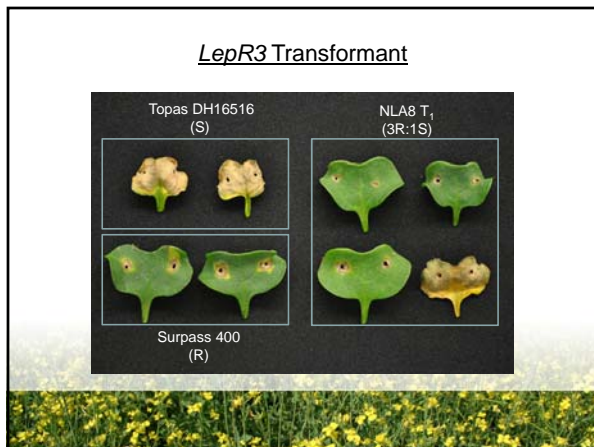


### LepR3 Candidate

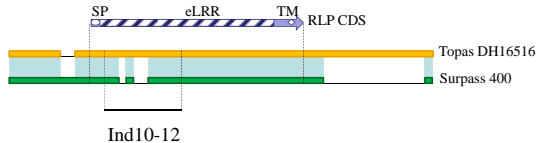
Candidate gene encoded a receptor-like protein

- ➔ Similar in structure to *Cf* RLPs for *Cladosporium fulvum* (leaf mold) resistance in tomato
- ➔ Large gene family; 56 members in *A. thaliana*, potentially 300+ in *B. napus*

Needed to devise a gene-specific cloning strategy



### LepR3 alleles: what is the function of transcripts in resistance and susceptibility?



Larkan et al. (2013): "Investigation of the LepR3 locus in the resistant 'Surpass 400' and the susceptible 'Topas DH16516' revealed three regions harboring insertion/deletion (indel) differences between the two loci [sic = alleles at one locus!]. However, these indels do not disrupt the expression of the two alleles as we were able to detect transcripts from both loci."



### The "mystery" of two R genes in Surpass 400

(as described in Larkan et al. 2013):

- "Van de Wouw et al. (2009) showed the *L. maculans* avirulence gene '*AvrLmS*' triggers a second *AvrLm1*-independent R-gene in 'Surpass 400' referred to as '*RlmS*.'"
- "Our results...corroborate the presence of both *AvrLmS* in *L. maculans* and *RlmS* in 'Surpass 400'"
- "...a recent survey of western Canadian *L. maculans* isolates ... 97.9% of the '*avrLm1*' isolates tested still produced a resistance response on 'Surpass 400' (Kutcher et al., 2010)." In other words, they contain *AvrLmS*.
- Most Australian isolates contain a virulence gene "*avrLmS*" which overcomes resistance in the "yet to be identified" *RlmS*
- Unfortunately also, for Australia, isolates with *avrLmS* (and *avrLm1*) were prevalent before the introduction of Surpass 400, therefore resistance was quickly "broken".
- Larkan et al. (2013): "Mapping of the *RlmS* resistance locus [in Surpass 400] and study of its interaction with *AvrLmS* isolates is currently in progress."

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