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Development of molecular markers for application in Australian Canola breeding (CMMP)

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Project update

- CMMP has made significant progress
  - Mapped DH populations for blackleg resistance
    - Skipton/Ag-Spectrum (Raman et al 2012)
    - Maxol/Westar (Raman et al 2013)
    - Columbus/Westar (Raman et al 2013)
    - BLN2762/Surpass400 (in preparation)
    - Ag-Castle/Westar-10 (in preparation)
- Developed molecular tools for molecular mapping of blackleg resistance loci, in collaboration with
  - UQ
  - DArT
  - UWA
  - AAFC, Saskatoon (Nick Larkan and Isobel Parkin)
- Accessed populations from national and International programs
  - China, Canada, France

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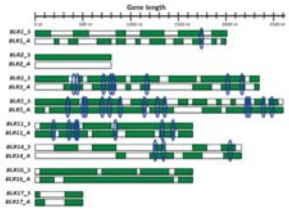
Plant Biotechnology Journal

Plant Biotechnology Journal (2012) **10**, pp. 709–715 doi: 10.1111/j.1467-7652.2012.00716.x

Identification and characterization of candidate *Rlm4* blackleg resistance genes in *Brassica napus* using next-generation sequencing

Reece Tollenaere[1], Alice Hayward[1], Jessica Dalton-Morgan[1], Emma Campbell[1], Joanne R.M. Lee[1], Michal T. Lorenc[2], Sahana Manoli[2], Jiri Stiller[2], Rosy Raman[3], Harsh Raman[3], David Edwards[2] and Jacqueline Batley[1,*]



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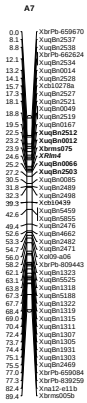

Saturated *Rlm4* locus with SNP markers in the DH population from Skipton/Ag-Spectrum

6K Infinium array was developed under ARC Linkage project (UQ-NSWDPI-Bayer-AGRF)

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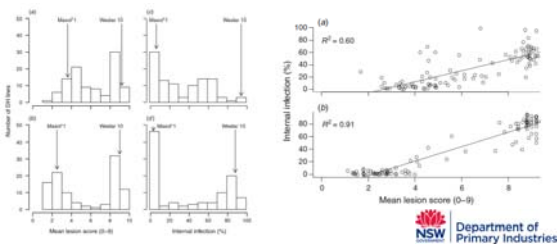
CSIRO PUBLISHING

Crop & Pasture Science, 2012, **63**, 1007–1017

http://dx.doi.org/10.1071/CP12255

Molecular mapping and validation of *Rlm1* gene for resistance to *Leptosphaeria maculans* in canola (*Brassica napus* L.)

Rosy Raman[A,D], *Belinda Taylor*[A], *Kurt Lindbeck*[A], *Neil Coombes*[A], *Denise Barbulescu*[B], *Phil Salisbury*[B,C], *and Harsh Raman*[A,D,E]



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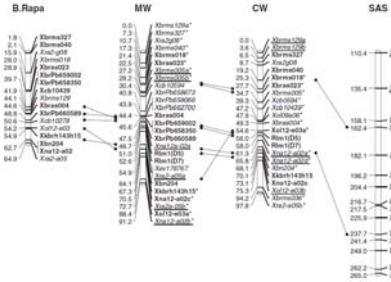
Comparative genetic and physical maps that show locations of molecular markers linked with *Rlm1* and *Rlm4*



Crop & Pasture Science, 2012, **63**, 1007–1017

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DNA Research Advance Access published December 22, 2011

DNA Research pp. 1–15, (2011) doi:10.1093/dnares/dsr041

Diversity Array Technology Markers: Genetic Diversity Analyses and Linkage Map Construction in Rapeseed (*Brassica napus* L.)

Harsh Raman[1,*], Rosy Raman[1], Matthew N. Nelson[2,3], M. N. Aslam[2], Rajeekaran Rajasekaran[2,4], Neil Wratten[1], Wallace A. Cowling[3,5], A. Kilian[6], Andrew G. Sharpe[7,†], and Joerg Schondelmaier[8]

- DArT Array (3,072 markers)
- Genome-wide markers (A, C genomes)
  - Diversity assessment
  - Linkage map construction
    - Lynx/Monty
  - Association mapping (ARAB Conference 2011)

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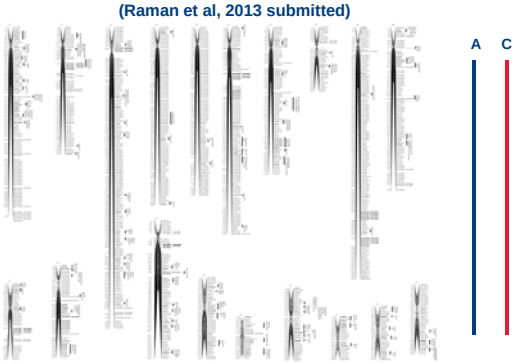
Developed consensus map based on DArT markers from six populations of canola and aligned with A and C genome scaffolds (Raman et al, 2013 submitted)



~1400 markers, ~1900cM

Genotyping by sequencing in canola

- Mapped three populations
  - Maxol/Westar
  - Ag-Castle/Westar
  - BLN2762/Surpass 400
- Developed genetic maps
  - Maxol/Westar and BLN2762/Surpass400
- Aligned genetic and physical maps
- Identified GBS marker associated with blackleg resistance in above three populations

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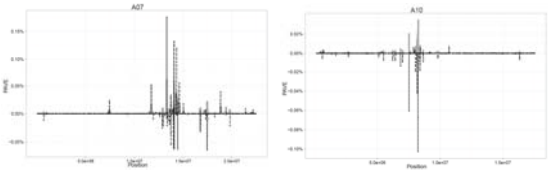
GBS based association analysis for blackleg resistance in BLN2762/Surpass400 population



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Validation and identification of superior alleles for blackleg resistance in diversity panel using GWAS

Phenotyping

- Cotyledon lesion
- Stem canker lesion
  - Field (2011)
  - Tub test
    - AV-Garnet (*Rlm1,9*)
    - Monola76 (*RlmS*)
    - ATR-Cobbler (*Rlm 4,9*)
    - CB-JardeeHT (*Rlm2,3*)
    - Hyola50 (Unknown)

Genotyping

- SSR
- DArT
- SNP
- GBS

Trait Analysis

- Population Structure
- Kinship
- TASSEL
- Statistical approaches

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Disease Resistance gene families in *Brassica rapa*

Index	Gene family	Number of genes
1	CC-NBS	14
2	CC-NBS-LRR	41
3	NBS	9
4	NBS-CC-NBS	1
5	NBS-LRR	20
6	NBS-LRR-TIR-NBS-LRR	1
7	TIR-NBS	23
8	TIR-NBS-LRR	90
9	TIR-NBS-LRR-NBS-LRR	1
10	TIR-NBS-LRR-TIR	2
11	TIR-NBS-LRR-TIR-NBS-LRR	1
12	TIR-NBS-TIR-NBS-LRR	1
13	TIR-NBS-X	1
14	TIR-Only	28
15	TIR-TIR	2
16	TIR-X	3

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Blackleg research in progress

DH Population	Phenotyping	Mapping
DHC2261/RR005	Field (2010) ACS (2012)	60K SNP
DHC2211/RP012*S	Field (2012)	60K SNP
08-6702P	Field (2012)	60K SNP
RP004/Ag-Outback	SSI/APR in 2013	60K SNP

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Beyond 2013 (-18)

- Map seedling and adult plant resistance genes in the Australian canola and related species
- VicDPI will phenotype all germplasm and make data available to marker program to identify molecular markers.
- Deliver markers in at least four mapping populations/year.
- Focus on homozygous germplasm

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Acknowledgments

Canola Molecular Marker Program

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UQ
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- Paul Eckerman (UA)

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- Bob Redden (VicDPI)

The University Of Queensland AUSTRALIA

- **Australian Canola Breeding Programs**
- Intl Blackleg Consortium, Canada
- HAU, China
- Isobel Parkin, AAFC Canada

GRDC

BioFirst

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