

NSW Department of Primary Industries

Development of molecular markers for application in Australian canola breeding (DAN117, 2013-14)

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Project update

Population	Phenotyping	Marker System	Status
Skipton/Ag-Spectrum	SSI Field (2010)	SSR, DA-T, SNP array	Theor Appl Genet 2012 Plant Biotech J (under review)
Maxol/Westar	SSI	SSR, DA-T, GBS	Crop & Pasture Research 2013
Columbus/Westar			
BLN2762/Surpass400	SSI Field	SSR, DA-T GBS	In preparation PLOS ONE
Ag-Castle/Westar-10	SSI		In preparation
DHC2261/RR005	Field (2010) ACS (2012)	60K SNP	In preparation
DHC2211/RP012-S	Field (2012)	60K SNP	In preparation
08-6702P	Field (2012)	60K SNP	Analysis in progress
RP004/Ag-Outback	SSI APR	60K SNP	In preparation
Skipton*2/Ag-Spectrum -BC,DH	Tub test (2013)	GBS (~7500 markers)	
11-5107	SSI Field (2013)-Wagga Field (2013)-Pacseed	GBS (~17000 markers)	In progress
Tapidor/Ningyou7	-	SSR, AFLP, genic, DA-T	NSW Department of Primary Industries

Blackleg scoring- Ergonomics

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Blackleg disease in Wagga Trials

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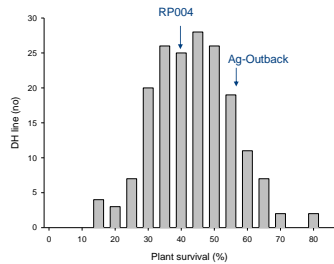
Blackleg disease in late sown trial at Wagga

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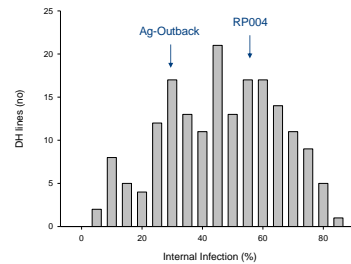
Phenotypic variation for resistance to *L. maculans* in RP04/Ag-Outback DH population

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Distribution of Plant Survival (%) in DH lines of RP004/Ag-Outback



Distribution of Internal Infection (%) in DH lines of RP004/Ag-Outback



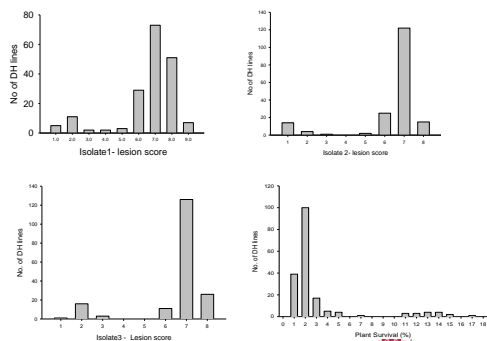
Progress on Blackleg-Hyola50 derived DH Populations

- From the parental screening, three isolates were selected to screen 11-5107 DH population (2012)
- 11-5107 population was phenotyped at VDEPI/Marcroft lab (2013)
 - Tub test and SSI
- 11-5107 and 11-5329 population were grown in blackleg nurseries
 - Pacific seed
 - Wagga (mixed stubble)
- Genotyping with GBS markers
- Map construction and QTL analysis

Phenotypic variation for resistance to *L. maculans* in 11-5107 DH population



Distribution of DH lines of 11-5107



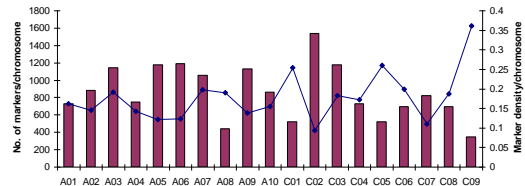
Correlation between cotyledon lesion score and plant survival (%) under field conditions of 11-5107 DH population

	Plant Survival (%)	Isolate 1	Isolate 2	Isolate 3
Plant Survival (%)	1			
Isolate 1	-0.8010073	1		
Isolate 2	-0.8659241	0.813	1	
Isolate 3	-0.8679098	0.827	0.897	1

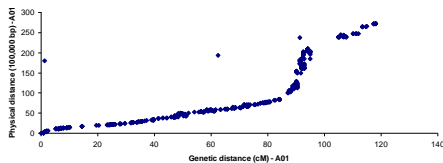
Genetic linkage map of 11-5107 DH population (Hyola50)

- DH lines: 169
- Genotyping platform:
 - Genotyping-by-sequencing (DArT-seq)
 - SNP
 - PAMs (*in silico* DArTs)
- Constructed linkage map with 17157 markers
 - Length of map: 2683.72 cM
 - Av. density: 1 marker/0.15cM

Features of genetic linkage map of 11-5107 (Hyola50 population)



Alignment of mapped GBS sequences on linkage map with reference A and C genomes



Mapped loci associated with resistance to *L. maculans* in 11-5107 DH population

- Whole genome map based approach
- Loci significantly associated with resistance were identified using phenotypic data generated
 - Field resistance
 - SSI
 - Tub test

Implemented newer marker technologies

- DArT arrays
 - Raman et al (2012), DNA Res 19(1):51-65
 - Raman et al (2013) BMC Genomics,14:277
- 6K SNP array
 - Raman et al, Plant Biotechnology Journal (under review)
- Genotyping by sequencing
 - Raman et al, PLOS One (under review)
 - ~16,774 markers mapped in BLN2762/Surpass population (3,041 SNPs and 13,733 PAMs)

Current strategy used for genotyping populations for blackleg resistance research

- DArT-GBS
- Mapped 7 populations segregating for resistance to *L. maculans*
 - BLN2762/Surpass 400
 - Maxol/Westar
 - Ag-Castle/Westar
 - Skipton/Ag-Spectrum (BC,DH population)
 - 11-5107
 - 11-5329
 - Tapidor/Ningyou7
- Developed genetic maps and currently constructing consensus map based on SSR, 60K SNP and GBS.
- Aligned genetic and physical maps of genomes A and C
- Identified/validating GBS markers associated resistance to *L. maculans*

GWAS for blackleg resistance

- Panel of 188 diverse lines
- Phenotyping
 - ascospore shower
 - SSI
- Genotyping
 - 89,618 polymorphic markers
 - 37,245 SNPs
 - 52,373 PAMs



GWAS-markers significantly associated with resistance to *L. maculans* (Hyola50 stubble)

Marker	Position	P-Value	$-\log_{10}(\text{P-Value})$	Proportion of Variance Explained
100038170	11910	8.76428E-06	5.057283661	0.111921669
3113874	43501	1.32575E-05	4.877536957	0.107662271
3087896	57501	1.46145E-05	4.835216538	0.106699572
100051562	7866	3.12347E-05	4.505362248	0.098908025
100034610	13066	4.38176E-05	4.358351359	0.095422964
100034610	13066	4.38176E-05	4.358351359	0.095422964
100022027	16572	5.11833E-05	4.290871381	0.09382086
3107917	46397	5.26038E-05	4.278982836	0.09353845
100005262	20404	5.48799E-05	4.260586366	0.093101358
100075665	2576	6.33017E-05	4.198584926	0.091627446



Genome-wide signals associated with adult plant resistance in Brassica



Acknowledgments

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