

Using Optimal Haploid Value Selection to Improve Blackleg Disease Resistance Traits in Canola

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Genomic Selection

- Useful for traits where variation is contributed by large number of loci, e.g. yield
- Large benefit for traits that are difficult or expensive to measure, or are measured late in breeding cycle
- Accelerate genetic gain by reducing generation interval, as well as increasing accuracy and intensity of selection

Consensus that genomic selection is simplest and most robust method for genomic-assisted breeding

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Canola Reference Populations

600+ spring and winter canola accessions assessed for multiple traits including blackleg, plot yield, height, flowering time and seed quality in replicated trials in Horsham and Lake Bolac area, Victoria

Field phenotyping

- Year 2015
 - 600 accessions in 2 locations 2 reps each
 - Traits: blackleg resistance, emergence, survival, internal infection
- Year 2016
 - 200 accessions in irrigated and rain-fed trials, 2-3 reps each
 - Agronomic traits measured
- Year 2017
 - 200 accessions in irrigated and rain-fed trials, 2-3 reps each
 - Agronomic traits measured
- Year 2018
 - ~1200 accessions (DHs and parents) in 1 location 2 reps (rain-fed)
 - Traits: blackleg resistance, emergence, survival, internal infection

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Phenotypes

- Phenotypes spatially corrected with AR1 models to account for in-field variability
- Traits
 - Blackleg disease
 - Adult plant survival, average internal infection
 - Agronomic traits
 - Emergence, vigour, lodging, flowering, plant height, maturity, yield
 - Seed quality
 - Arachidic, eicosenoic, glucosinolates, linoleic, linolenic, oleic, palmitic, stearic acids, oil and protein content, moisture

Genomic heritability

- Spring lines

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Genotyping

Transcriptome genotyping-by-sequencing

- Protocol aimed at delivering SNP genotyping at minimal cost per sample
- Reduced cost of sequencing library prep through volume reduction
- Genome complexity reduction
- Open platform does not require manipulation of restriction enzymes or primers
- All SNPs are genic

Genotyping-by-sequencing (GBS)

- 488 spring and winter accessions
- Call SNP in RNA sequence from leaves
- ~60,000 to 90,000 high quality SNP

Genotyping-by-sequencing through transcriptomics: implementation in a range of crop species with varying reproductive habits and ploidy levels

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Genomic Selection Methods

Genomic relationship based on SNP markers

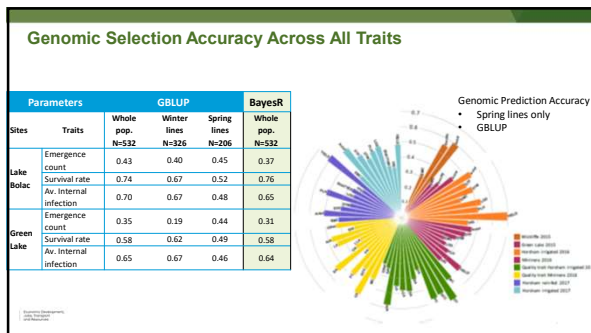
- Genomic Best Linear Unbiased Prediction (GBLUP)
 - Genomic relationship matrix
- BayesR
 - Bayesian genomic selection method
- BayesRC
 - Bayesian genomic selection method that can use prior knowledge on QTL (biological priors)

Cross validations: 10 fold CV for the whole set and winter lines, 5 fold for spring lines.

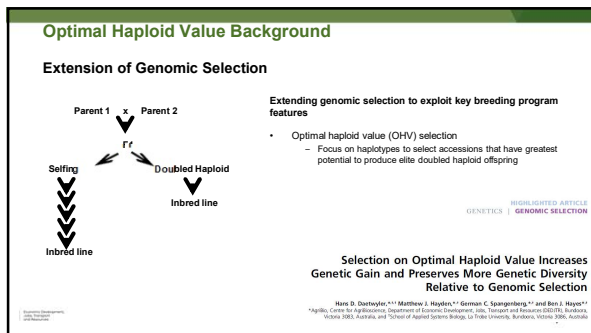
Genomic Prediction Using Prior Quantitative Trait Loci Information Reveals a Large Reservoir of Undetected Blackleg Resistance in Diverse Canola (*Brassica napus* L.) Lines

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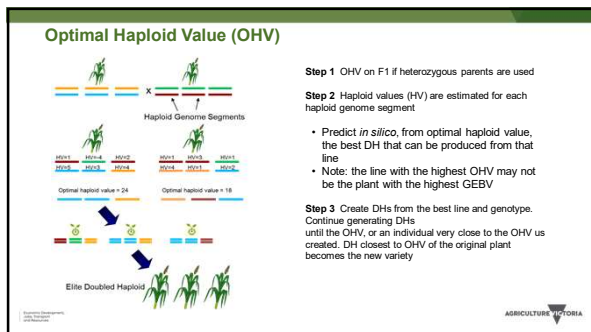
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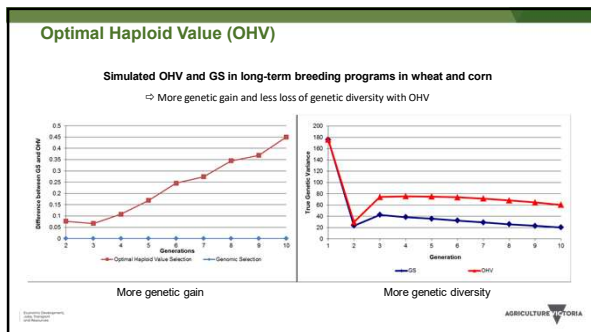
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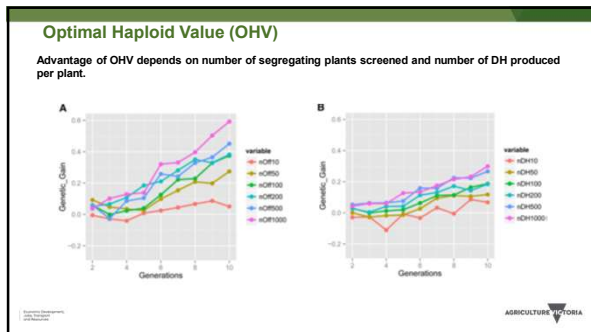
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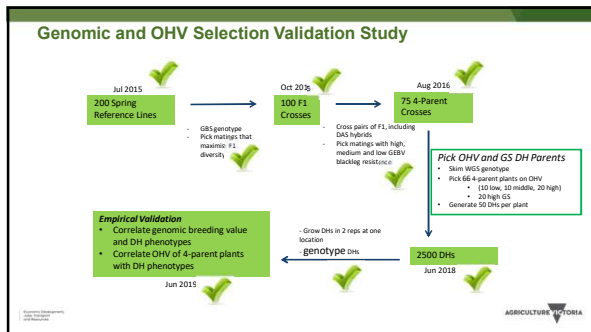
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OHV Validation Study

Methods

- Survival marker effects for GEBV and OHV calculation estimated using BayesR and 2015 blackleg field trials
- Overlap of GBST and SWGS markers then imputed and phased (Beagle and Eagle)
 - ~30,000 SNP
- 4 groups of DH parents chosen
 - High OHV, High GEBV, Medium OHV, Low OHV
 - Considerable overlap in high groups
- DHs, DH parents, and original parents planted in blackleg nursery
 - 1 location with 2 reps per entry
 - Collected emergence, survival and internal infection phenotypes
- Survival phenotypes spatially corrected with AR1 model
 - BLUPs used as adjusted phenotypes


OHV Validation Tests

Phenotypic comparisons (survival)

- Difference between OHV-H, OHV-M, and OHV-L sets
- Difference between OHV-H and GEBV-H sets
- Top DH, groups means, meanTop10
- Genetic gain

Comparisons of phenotypes with GEBVs

- Accuracy of GEBVs
 - Original parents
 - DHs
 - Across all sets and with each




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OHV Validation Study

Number of DHs per parent plant in field trial

- Aim was 50 DHs per plant

- Number DHs
 - Mean number of DHs in GEBV-H was 18.5 (range 1 – 46)
 - Mean number of DHs in OHV-H was 23.4 (range 1 – 90)
- Correlation of number of DH progeny and Survival BLUPs
 - GEBV-H: 0.66
 - OHV-H: 0.53
 - Larger DH progeny groups had higher GEBVs and OHVs
 - A limiting factor for validation study
- Reasons for fewer than 50 DHs produced
 - Some plants not amenable to DH production
 - Not all DHs produced seed or enough seed for field trial




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OHV Validation Study

Set	Mean BLUP	StDev BLUP	Top BLUP	Name or Parents of Top Line	Mean Top10 BLUE	Genetic Gain Top10
Original Cultivars	51.80	19.07	90.32	CG5906	78.73	-
GEBV-H	64.47	15.09	90.87	VIC015/AV-RUBY//VIC048/ATR-STINGRAY	87.86	11.6%
OHV-H	62.86	17.90	93.00	VIC005/AV-JADE//VIC048/RT06	87.72	11.4%
OHV-M	51.34	16.72	63.84	-	56.03	-28.8%
OHV-L	30.61	16.74	45.46	-	39.27	-50.1%

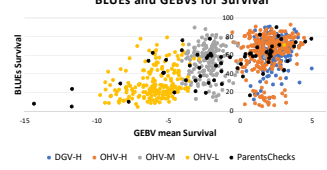
- Highest survival was a DH from OHV
- Mean of Top10 DHs equal between OHV-H and GEBV-H sets
- Standard deviation of OHV-H set higher than GEBV-H set
- Clear differentiation between OHV-H, OHV-M, and OHV-L sets




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OHV Validation Study

BLUEs and GEBVs for Survival



- Equal performance of GEBV-H and OHV-H sets
- OHV-H set more variable than GEBV-H set
- Clear differentiation between OHV-H, OHV-M, and OHV-L sets




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OHV Validation Study

Set	GEBV Acc Green Lake	GEBV Acc Wyckliffe	GEBV/mean Acc Both sites
Original Parents	0.60	0.66	0.66
All DH sets	0.59	0.61	0.61
GEBV-H	0.14	0.05	0.11
OHV-H	0.15	0.14	0.16
OHV-M	0.14	0.02	0.09
OHV-L	0.17	0.41	0.32

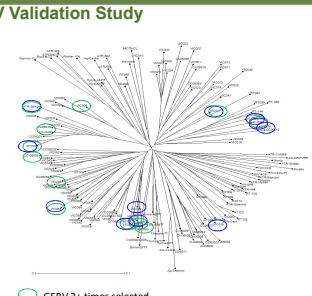
*GEBV accuracy = correlation of BLUPs and GEBVs

- Accuracy of GEBVs in original parents high
- Accuracy across all DHs also high
- Accuracy in DH sets reduced especially high and medium sets
 - Sets are highly selected
- Accuracy of OHV-L set higher than other sets




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OHV Validation Study



Original Parent	OHV	GEBV
VIC054	6	4
AV-RUBY	5	2
RT076	5	6
VIC058	5	6
ATR-STINGRAY	4	3
VIC007	4	2
AV-SAPPHIRE	3	2
RT001	3	1
RT078	3	2
VIC015	3	0
VIC017	3	3
VIC029	2	4
ATR-SUMMITT	2	2
AV-JADE	2	7
CG5906	2	4
RAINBOW	2	1
RT059	2	2
VIC005	2	4
VIC008	2	4
VIC018	2	2
VIC049	2	3
VIC052	2	2
VIC059	2	1
CG5915	1	0
MUSTER	1	0
RT010	1	0
RT096	1	0
VIC010	1	1
VIC027	1	0
RT148	0	2
VIC003	0	1
VIC006	0	1
Number of Unique Parents	29	26
Children (Summed)	13	18



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Summary

- Reference populations implemented for key traits including blackleg resistance
- Genomic prediction accuracy at a level that allows immediate implementation of genomic selection
- Genomic and OHV selection equal genetic gain
 - Likely due to lowish DH number per family achieved
 - Confirmed trends in simulations
- OHV selection results in more diverse breeding population
 - Important for long-term genetic gain
 - More prediction power for genomic predictions?
- Balance between quantitative and qualitative resistance for blackleg
- Exciting time in canola breeding!

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Publications to Date

In Review:

Fikere et al. 2018:
 Meta-Analysis of GWAS in Canola Blackleg (*Leptosporium anisopliae*) Disease Traits Demonstrates Increased Power from Imputed Whole-Genome Sequences. *Plant Biotech Journal*

Fikere et al. 2018:
 Genomic Prediction and Genetic Correlation of Agronomic, Blackleg Disease, and Seed Quality Traits in Canola (*Brassica napus* L.). *Frontiers in Plant Science*

Genotyping-by-sequencing through transcriptomics: implementation in a range of crop species with varying reproductive habits and ploidy levels

Diversity and Genome Analysis of Australian and Global Oilseed Brassica napus L. Germplasm Using Transcriptomics and Whole Genome Re-sequencing

Evaluation and Recommendations for Routine Genotyping Using Skim Whole Genome Re-sequencing in Canola

Genomic Prediction Using Prior Quantitative Trait Locus Information Reveals a Large Reserve of Underutilized Blackleg Resistance in Diverse Canola (*Brassica napus* L.) Lines

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