DISCOVERY AND APPLICATION OF DNA MARKERS FOR RESISTANCE TO *TERATOSPHAERIA* IN *EUCALYPTUS GLOBULUS*

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Background

- Teratosphaeria leaf disease (TLD; formerly Mycospaerella leaf disease or MLD) is one of the most prevalent foliar diseases of eucalypts
- Plantation eucalypts particularly susceptible
- TLD is widespread in *Eucalyptus globulus* growing areas of WA, VIC and Tasmania
- Routine phenotypic screening for the disease is difficult due to the lack of established screening methods
- Identification molecular markers associated with TLD is attractive as markers can be used for screening the seedlings to identify resistant lines

Materials and Methods

- The main aim of this study is to identify markers associated with TLD and test genomic selection models for application in breeding programs
- Two base populations in Tasmania (Salmon River and Temma) and three trials from WA (Towes, Montes and Sinclair) are used in this study
- Potential markers linked to disease resistance were obtained by sequencing resistance and susceptible pools
- We used our new genotyping method developed in-house to genotype these SNPs
- 48 resistant and susceptible trees from the two base populations from Tasmania and the two trials from WA were genotyped with the marker panel

Markers associated with TLD resistance

- Identified 240 candidate SNPs associated the disease through sequencing

- One of the advantages of our new genotyping method is identification of additional markers from the selected SNP regions

- More than 2000 SNPs were genotyped. After filtering for minor allele frequency and SNP call rates, 650 markers were selected for further analyses

- Association analyses revealed 69 significant SNPs associated with TLD resistance

Markers associated with TLD resistance

variant	P(R)	Gene_ID	Effect	Annotation
SNP	1.50E-05	Eucgr.K03036	DOWNSTREAM: 527 bases	glutathione S-transferase TAU 8
SNP	0.0001	Eucgr.A01622	DOWNSTREAM: 200 bases	C2H2-type zinc finger family protein
SNP	0.000182	Eucgr.K03036	DOWNSTREAM: 501 bases	glutathione S-transferase TAU 8
SNP	0.00034	Eucgr.K01151	INTRON	WRKY DNA-binding protein 69
SNP	0.000529	Eucgr.C02602	INTRON	glutathione peroxidase 1
SNP	0.000755	Eucgr.F01014	INTRON	NB-ARC disease resistance protein
SNP	0.000887	Eucgr.D00728	SYNONYMOUS_CODING	Disease resistance protein (TIR-NBS
SNP	0.001115	Eucgr.D00728	SYNONYMOUS_CODING	Disease resistance protein (TIR-NBS
SNP	0.001705	Eucgr.J03136	SYNONYMOUS_CODING	spermidine hydroxycinnamoyl transfe
SNP	0.002975	Eucgr.H02576	UTR_3_PRIME	glutathione S-transferase tau 7
SNP	0.003056	Eucgr.D01857	DOWNSTREAM: 17 bases	glutathione peroxidase 6
SNP	0.00328	Eucgr.G00887	SYNONYMOUS_CODING	NB-ARC domain-containing disease
SNP	0.003524	Eucgr.D01857	DOWNSTREAM: 18 bases	glutathione peroxidase 6
SNP	0.003581	Eucgr.G00887	SYNONYMOUS_CODING	NB-ARC domain-containing disease
SNP	0.004148	Eucgr.J02089	SYNONYMOUS_CODING	Disease resistance-responsive family
SNP	0.004898	Eucgr.K03036	DOWNSTREAM: 477 bases	glutathione S-transferase TAU 8
SNP	0.005332	Eucgr.J03136	INTRON	spermidine hydroxycinnamoyl transfe
SNP	0.0062	Eucgr.D00730	NON_SYNONYMOUS_CODING	Disease resistance protein (TIR-NBS
SNP	0.007304	Eucgr.G00690	UPSTREAM: 377 bases	NB-ARC domain-containing disease

Predicting resistance to TLD

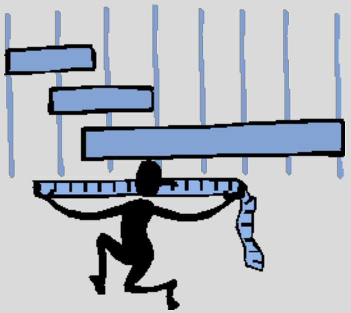
- While association and QTL studies are useful for identification of significant markers, individual marker effect is too small to be useful in breeding programs
- Instead of using significant markers individually, marker effects from several markers can be combined for predicting traits – genomic selection
- Traits estimated by just using marker data are marker breeding values (MBVs) or genomic estimated breeding values (GEBVs)

MBV estimation

Training Population

DNA marker data

Phenotypic data

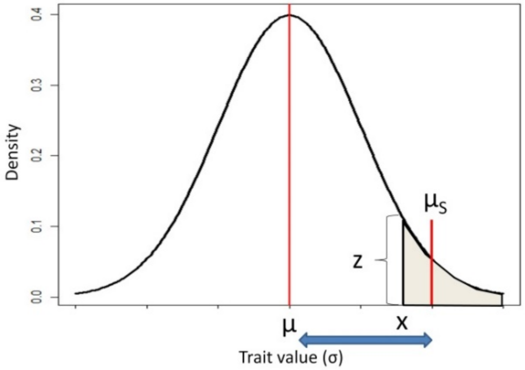


Model training

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}$$

Testing Population

Predict and select



Selection candidates

Accuracy = correlation between predicted and true values

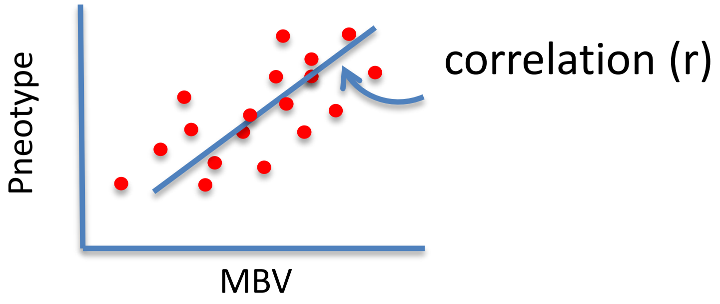
MBVs: marker based breeding values

Testing marker predictive ability

Based on DNA alone we predict the trait in trees that already have accurate phenotypes

Predictive ability

The correlation (r) between our marker predictions (MBVs) and phenotypic measurements in a modest number of trees



Accuracy

Accuracy of phenotypic selection

$\surd$ heritability (h^2) or h

Accuracy of marker based selection = predictive ability divided by the square root of the heritability of the trait.

$$\frac{\text{Predictive ability (r)}}{\surd\ \text{heritability } (h^2) \text{ or } h}$$

Predictive ability of markers

Train	**Test**	**Predictive ability**
SR_TE_TW	MT	0.28
TW	MT	**0.33**
SR	MT	0.14
TE	MT	0.01
SR_TE_MT	TW	**0.61**
MT	TW	**0.62**
SR	TW	0.05
TE	TW	**0.30**
SR	TE	0.00
MT	TE	0.10
TW	TE	0.06
MT_SR_TW	TE	0.10
MT	SR	0.19
TE	SR	0.05
TW	SR	0.08
MT_TE_TW	SR	0.11

Highest with MT as training and TW as testing

MT and TW are CP families while SR and TE are OP families

Significance of these results

- Accuracy of GS models is influenced by marker-trait association and the pedigree relationships captured by the markers
- High accuracies are expected when training and testing populations are closely related
- High accuracies observed with unrelated training and testing populations indicate marker-trait associations are contributing to high accuracies

Application in breeding populations

- These results indicate that the markers developed in this study can be used for screening for disease resistance
- Disease prediction models can be developed using seed orchard and progeny tested trees as training populations
- MBVs of TLD for seed orchard trees can be estimated with progeny trials assessed for the disease
- Prediction model can be used for estimating MBVs of the seedlings and selecting for resistant lines

Summary

- Identified several marker significantly associated with TLD resistiantance
- Developed a panel of markers that can be used for screening for disease resistance
- High accuracies were observed in breeding trials even when training testing populations are unrelated
- Disease resistance markers from this study can be combined with other trait marker panels for screening and selecting different traits simultaneously

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