

**Characterization of phenotypic and  
genotypic selection for simple and  
complex traits of barley  
(*Hordeum vulgare* L.)**

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## **Declaration**

I declare that the research presented in this thesis is original and was undertaken and written by myself, except where specifically indicated in the text.

The thesis has been completed during the course of enrolment in a PhD degree at Murdoch University and has not been used previously for a degree or diploma at any other institution.

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Reetinder Gill

## Abstract

The challenge to modern day plant breeding is to constantly strive to improve the efficiency and efficacy of utilising the available resources. This requires the integration of conventional breeding technologies together with molecular genetic markers to significantly improve the breeding programs.

Male sterile facilitated recurrent selection (MSFRS) can improve a breeding program by reducing the variety release time to almost half, maintaining continued out-crossing and recombination, thus broadening the germplasm base. A molecular genetic marker system for the male sterile-orange lemma-shrunken endosperm (*msg6-rob1-sex1*) linkage block was developed during this study, to aid in the differentiation of fully fertile homozygous from fully fertile heterozygous plump individuals in F<sub>2</sub> populations. These individuals may be desired for the production of doubled haploids or for use as pollen donor parents in further cycles of MSFRS. A DF578/6\*Gairdner BC<sub>5</sub> population was chosen for the study and SSR markers were identified in the region of the *msg6-rob1-sex1* linkage group on the short arm of chromosome 6H. A linkage map was constructed and it was found that microsatellite markers HVM65, HVM74 and Bmgttttt1 are tightly linked to *msg6-rob1-sex1* linkage block.

Within the MSFRS process, it was found that genotypic selection with SSR markers is an advantage when the phenotype of interest is controlled by a single major gene and the marker is either “perfect” or closely linked. Genotypic selection was found to be very effective in selecting for the aluminium tolerant genotypes with the SSR marker HVM68,

where aluminium tolerance is known to be controlled by *Alt* or *Alp* gene on chromosome 4H.

For the complex trait such as barley scald resistance, genotypic selection was found to be of limited value. Simulation studies demonstrated that multiple genetic factors need to be taken into account while selecting for a complex phenotype. As expected, phenotypic selection method was found to be efficient in selecting for scald resistance as it selected minor genes along with two known major genes for scald resistance on chromosome 4H (*Rhs<sub>Vlamingh</sub>*) and 6H (*Rhs<sub>WABAR2147</sub>*).

Simulation studies based on the experimental results provided a guide for the frequency and timing of the use of molecular markers in the breeding program. It was found that markers that are loosely linked to the gene of interest should only be used once early in the breeding program. As in the case of scald resistance, where the markers-gene distances are 5cM or 30 cM, the genes can only be partially fixed. Further cycles of genotypic selection for scald resistance will lead to the selection of the susceptible genotypes instead of resistant genotypes as the phenotype comes in repulsion rather than the coupling phase with the scald resistant genes.

GGT (an acronym of Graphical GenoTypes) software package was extensively used to study genotypic changes in response to selection and to select for the genotypes carrying resistant genes of interest. Based on the allele frequencies and the marker-scald associations carried out using GGT, the SSR marker Bmac213 (1H) was found to be associated with powdery mildew (*Mla<sub>WABAR2147</sub>*) resistance and SSR markers GBM1221

(4H) and Bmac316 (6H) were found to be associated with scald (*Rhs<sub>v</sub>lamingh* and *Rhs<sub>WABAR2147</sub>*) resistance.

Results presented in this thesis have enabled the identification of mechanisms behind the success of phenotypic selection and its use while selecting for quantitative traits as it incorporates the minor gene effects while selecting for the major genes. Genotypic selection method was found to be efficient in selecting for the desired genotypes but may not give the desired result in terms of phenotype when a complex trait is involved. Genotypic selection will be at par with the phenotypic selection for the complex quantitative traits if the associated markers with the minor genes are included in the selection. Both phenotypic and genotypic selection methods together can be used effectively in the breeding program to increase the rate of genetic gain.

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Finally, I would like to thank my parents and my brothers, Puneet and Sumeet, who have always been there for me as an unwavering support.



## **Dedication**

I wish to dedicate this thesis to my daughter, Raavi - born in March 2009 - who is too young to understand this but I hope this will inspire her to achieve her goals under any circumstances. One of the best experiences that I and Mandeep lived through in this period was waiting for our first child to be born and our little princess has provided an additional and joyful dimension to our life.

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- 4.2 An overview of the models fitted to the leaf scald and powdery mildew data collected three times during the season, for both the crosses and each cross with both the treatments. The numbers represent the autoregressive correlation coefficients for natural spatial variation along with standard errors (SE) for the estimates. Where: T1 – phenotypic selection (Treatment1), T2 – genotypic selection (Treatment2).
- 4.3 An overview of the models fitted to leaf area damage due to scald. Variations are based on the data collected for both the crosses with both the treatments. Where: T1 – phenotypic selection (Treatment1), T2 – genotypic selection (Treatment2), lin – linear, col – column and AR - autoregressive.
- 4.4 Additive effect sizes of the genes under study along with the additional minor QTLs for scald, for all the genotypes. Where 11 – both the alleles from Vlamingh, 12 – one allele from Vlamingh and one from WABAR2147 and 22 – both the alleles from WABAR2147.
- 4.5 Details of the markers used for genotypic selections for powdery mildew (*Mla*<sub>WABAR2147</sub>) resistance and scald (*Rhs*<sub>Vlamingh</sub> and *Rhs*<sub>WABAR2147</sub>) resistance for Cross 1 and Cross 2 populations.
- 4.6 Significance (p-values) of Time, Family and Time\*Family, in the repeated measurement analysis of both the crosses with two treatments each. Where T1 –

phenotypic selection (Treatment1), T2 – genotypic selection (Treatment2), SC1 – scald score 1, SC2 – scald score 2 (taken 30 days after SC1) and SC3 – scald score 3 (taken 30 days after SC2).

- 4.7 Correlation coefficients between the three scald scores for both the Crosses and Treatments. T1 – phenotypic selection (Treatment1), T2 – genotypic selection (Treatment2), SC1 – scald score 1, SC2 – scald score 2 (taken 30 days after SC1) and SC3 – scald score 3 (taken 30 days after SC2).

## **Chapter 5:**

- 5.1 Expected genotype frequencies based on the pedigree of the population used for graphical genotyping for scald and powdery mildew resistances.
- 5.2 Details of the SSR markers used for graphical genotyping.
- 5.3 Allelic codes assigned to the Parents and their different combinations for graphical genotyping display. Different combinations are based on the five parents; A-WABAR2096, B-WABAR2147, C-Birka, D-Skiff and E-Vlamingh.
- 5.4 Categorizing the alleles into parental alleles according to Identity by Descent. Allele codes are based on Table 5.3.
- 5.5 Cumulative allele frequencies of all the alleles present in the graphically genotyped population for scald and powdery mildew resistances. For allele codes please see Table 5.3.
- 5.6 The genotypes with their genotypic frequencies for both phenotypically selected

(Treatment 1) and genotypically selected (Treatment 2) populations, based on Figure 5.8.

- 5.7 The frequencies of the scald resistant genes and their associated markers at 30cM and 5cM distances, for 0, 1, 2, 3 cycles of selection. The table is based on the frequencies from Figure 5.10. Where: PS – phenotypic selection (Treatment 1) and GS – genotypic selection (Treatment 2).
- 5.8 The frequencies of the favourable alleles after one cycle of selection, observed in the present study and the one simulated using Qu-Gene have been compared. This table is based on Figure 5.4, Figure 5.5 and Table 5.7.