# Applications and challenges of marker-assisted selection in the Western Australian Wheat Breeding Program

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## ABSTRACT

Molecular markers are being increasingly used for the deployment of multiple genes for disease, quality and agronomic traits. To cost effectively utilize molecular markers, a communication and management structure between the molecular team and breeding team must be established. The needs of each group must be discussed periodically. For the effective use of markers, breeders require timely delivered accurate and reliable information. This presentation aims to outline; (1) implementation of molecular markers for the breeders' traits, and (2) discussions about the high-throughput and logistics of marker-assisted selection (MAS) applications in a large breeding program.

## INTRODUCTION

Molecular marker technologies offer a range of opportunities to plant breeders to improve the efficiency of breeding programs. The deployment of multiple genes relevant to a given environment is the major aim of a breeding program. Successful applications of marker-assisted selection (MAS) have been reported in both wheat (Cakir *et al*. 2003, Kuchel *et al*. 2007) and barley (Rae *et al*. 2007). Recent development of Multiplex-Ready Technology (MRT) has enabled the selection of multiple traits with multiple markers (Cakir *et al*. 2007 Hayden *et al.* 2007 and Cakir *et al*. 2008) with reasonable increase in high througput.

Western Australian wheat breeding program, recently re-established as a commercial company under the trade name of InterGrain, has three subprograms. Each sub-program is led by a breeder and is responsible for developing varieties for their respective environments. Molecular marker laboratory services all three sub-programs simultaneously and this requires a good planning and management structure. This study summarises the marker applications and discusses the high-throughput and logistics of MAS applications in a large breeding program.

## MATERIALS AND METHODS

### *Sampling and DNA preparation*

All the lines to be tested are grown in the glasshouse or in the field and 2-3 cm leaf tissues are sampled into 96 deep welled PCR plates (Interpath services). Excel based transfer files are used to define the requirements for each project or screening. Each breeder is given a code such as X, Y, W to identify and catalogue the transfer files.

Genomic DNA was extracted in 96 well plates based on the method of Edwards *et al*. (1991). Briefly, 250ml of extraction buffer I (200mM Tris-HCl pH 7.5, 250mM NaCl, 25mM EDTA) and a single stainless steel ball bearing are added into deep well PCR plates. Samples are then disrupted by shaking at 500 strokes/minute for 1.5 minutes using a Geneogrinder 2000 (CertiPrep). SDS is then added to 90ml of the extraction mix to a final concentration of 0.5% and the samples are incubated at $65^0$C for 1 hour. The DNA is then precipitated using ethanol and resuspended in 100ml of TE buffer (10mM Tris, 0.1mM EDTA). BioMek 3000 (Beckman) robot is used for setting up PCRs in 384 well formats.

### *Fragment analysis and reporting the results*

Fragment analysis of PCR products are performed by running either agarose or acrylamide gels or non-gel based capillary analysis using ABI3730XL. While simple presence/absence type of markers are analysed with agarose gels, fragment analysis of SSR markers is carried out using either acrylamide gels or MRT as described by Hayden *et al.* (2007). Results are recorded on the transfer files and placed in a server where each breeder could access.

## PROGRESS AND DISCUSSION

Molecular marker development and validation are fully integrated with the breeding operations, and activities are focused on the traits that breeders prioritise in the program. As new markers are identified from our work and/or literature these are integrated into marker cassettes formed for different group of traits (i.e. quality, disease) for their effective use in MAS. Based on the traits that are required by the breeding program we have established a trait-based map that serves as a guide to breeders in their daily activities including crossing decisions. The program currently has the capacity of using MAS for 42 traits/genes (Table 1). To define the needs of three sub-programs and for the timely delivery of the outputs by the molecular lab, the use of excel based data base has allowed us to keep track of the requirements of each of the three sub-programs to make sure that the marker laboratory spends its resources equally. Data analysis is one of the limitations of high-throughput marker applications and to overcome this additional analysis tools are being developed.

**Table 1. The list and chromosome locations of markers/traits.**

| | Marker/trait | Chro. loc | | Marker/trait | Chro. loc |
|---|---|---|---|---|---|
| 1 | Bx7 | 1BL | 22 | Lr47 | 7AL |
| 2 | Bo1 | 7BL | 23 | Lr9 | 6BL |
| 3 | Flour Color | 7AL | 24 | PHS | 3BL |
| 4 | Al(Almt1) | 4DL | 25 | PHS | 4AL |
| 5 | BGGP(protein) | 6BS | 26 | PinA | 5DS |
| 6 | 1B/1R | 1BL | 27 | PPO | 2AL |
| 7 | BYDV | 7DL | 28 | PPO | 2DL |
| 8 | Cre 1 | 2BL | 29 | Rht1 | 4BS |
| 9 | Cre3 | 2DL | 30 | Rht2 | 4DS |
| 10 | GBSS Null4A | 4AL | 31 | Rht8 | 2DS |
| 11 | GBSS Null7A | 7AS | 32 | Sr2 | 3BS |
| 12 | GBSS Null7D | 7DS | 33 | Sr22 | 7AL |
| 13 | Imi B8 | 6D | 34 | Sr26 | 6AS |
| 14 | Imi FS2 | 6DL | 35 | Sr30 | 5DL |
| 15 | Imi K42 | 6A | 36 | Sr32 | 2BL |
| 16 | LMA | 7BL | 37 | Sr33 | 1DS |
| 17 | Lr13/Lr23 | 2BS | 38 | Sr36 | 2BS |
| 18 | Lr19/Sr25 | 7DL | 39 | VPM | 2AS |
| 19 | Lr24/Sr24 | 3DL | 40 | Yellow Spot | 5BL |
| 20 | Lr34/Yr18 | 7DS | 41 | Yr10 | 1BS |
| 21 | Lr46/Yr29 | 1BL | 42 | Yr34 | 5AL |

**Table 2. Progress in the MAS program in the last five years.**

| Year | No. of traits/genes | No. of assays |
|---|---|---|
| 2003 | 13 | 13277 |
| 2004 | 17 | 14888 |
| 2005 | 24 | 23254 |
| 2006 | 38 | 28385 |
| 2007 | 42 | 35000 |

***Progress in MAS***

The program has steadily increased the use of MAS in the last five years from about 13000 to 35000 (Table 2). These improvements were mainly due to:

- Establishment of excellent working relationship between molecular staff and breeding staff
- Better management of lab staff
- Development of transfer files for processing of samples and reporting results
- Establishment of a plate-based sampling and improvement in DNA extraction protocols
- Increase use of Multiplex Ready Technology (MRT)
- Development of "Marker Wizard", an excel based database, for achieving:
    - A quota system for 3 sub-breeding group (long season, short season and soft wheat)
    - Identifying peak and off-peak periods
    - Integrating a weighing factor for each trait analysis based on the complexity of the trait and or/marker

***MAS applications***

MAS and more traditional screening methods are seen as complementary, and both are utilised depending on the breeding generation.

*Marker assisted selection is applied in four major areas as follows:*

1. *Parent selection from complex cross $F_1$s and heterogeneous donors*

Seventy-percent of MAS applications is for the selection of parental lines in crossing programs, particularly screening of $F_1$s in backcross and top-cross situations. The efficiency over conventional crossing strategies is the greatest where crossing targets multiple traits, and particularly in tracking recessively inherited traits where the need for progeny testing is removed.

2. *Pyramiding Resistance genes.*

MAS for pyramiding genes is especially used for rust and herbicide tolerance genes. The wheat breeding program currently has a major effort in upgrading resistance to the three rusts. Future aims to produce cultivars with multiple effective genes to each of the three rusts to reduce the risk of development and multiplication of new rust races via simple step-wise mutations or somatic hybridization, resulting in loss of effectiveness of resistance genes.

3. *Population enrichment in accelerated breeding systems*

The DAFWA breeding program has integrated MAS in both the $F_2$ bulk progeny and single seed descent breeding systems. The program has achieved the greatest efficiency by selection as early as possible in the breeding process, but this is also where number of individual lines is the greatest. As the affordability, throughput and range of assays improves the amount of screening the breeding program can do in $F_2$ and $F_3$ generations, and in the $F_5$ and $F_6$ generations after re-selection will increase significantly. Effective selection at early generations increases the overall efficiency of the breeding process as lines entering expensive replicated, multi-site yield trials are more targeted and have a greater probability of success.

4. *Marker facilitated selection for backcross breeding*

Currently MAS program is being used to trace a gene from a donor parent in a backcross population rather than selection for the recurrent parent using a whole genome selection approach. Although breeders have considered the use of MAS in accelerated backcrossing by selection for the recurrent parent background genotype, at this stage, the high cost of whole genome screening and the need to handle larger numbers of plants within a substantial backcrossing program has resulted in the decision to not use this strategy within the breeding program. However, background selection is expected to be possible as more SNP type markers are available for MAS.

## CONCLUSIONS

Transition of markers from mapping populations to implementation in a large breeding program is proving to be a considerable challenge to both breeders and molecular biologists. This can be attributed to the significantly greater number of assays required by a breeding program, and the more complex crossing structure used in a breeding program compared with the simple crosses between two fixed line parents used for most experimental populations. Timely delivery of results is also a significant issue to the breeding program. Late arrival of marker information in a large backcrossing program creates immense problems and additional work for the breeding program. As the costs reduce, and throughput and range of assays improves the amount of screening carried out in the breeding program will continue to increase. Good data handling and laboratory management systems become essential to handle the larger numbers of lines and assays required by a breeding program.

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