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Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy

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Abstract

The advent of molecular markers as a tool to aid selection has provided plant breeders with the opportunity to rapidly deliver superior genetic solutions to problems in agricultural production systems. However, a major constraint to the implementation of marker-assisted selection (MAS) in pragmatic breeding programs in the past has been the perceived high relative cost of MAS compared to conventional phenotypic selection. In this paper, computer simulation was used to design a genetically effective and economically efficient marker-assisted breeding strategy aimed at a specific outcome. Under investigation was a strategy involving the integration of both restricted backcrossing and doubled haploid (DH) technology. The point at which molecular markers are applied in a selection strategy can be critical to the effectiveness and cost efficiency of that strategy. The application of molecular markers was considered at three phases in the strategy: allele enrichment in the BC_1F_1 population, gene selection at the haploid stage and the selection for recurrent parent background of DHs prior to field testing. Overall, incorporating MAS at all three stages was the most effective, in terms of delivering a high frequency of desired outcomes and at combining the selected favourable rust resistance, end use quality and grain yield alleles. However, when costs were included in the model the combination of MAS at the BC_1F_1 and haploid stage was identified as the optimal strategy. A detailed economic analysis showed that incorporation of marker selection at these two stages not only increased genetic gain over the phenotypic alternative but actually reduced the over all cost by 40%.

Abbreviations: AGT – Australian Grain Technologies Pty Ltd; DH – Doubled haploid; ET – Environment type; HMW – High molecular weight; MAS – Marker-assisted selection; TPE – Target population of environments

Introduction

The genetic improvement of inbred crops through plant breeding is a proven and successful route to increased production efficiency within agricultural systems (Allard 1960). In Australia, wheat breeders have placed particular emphasis on improving the adaptation (ability to produce high grain yield across multiple and varied environments) and the bread making quality of wheat (Hollamby et al.

1983). However, reducing the potential impact of foliar pathogens, particularly leaf rust (*Puccinia triticina* f. sp. *tritici*), stem rust (*Puccinia gramminis* f. sp. *tritici*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*), through selection for adult plant resistance genes (believed to be durable) has also helped provide protection from the rapid development of varietal susceptibility that plagued farmers in the first half of the 20th century (McIntosh 1992). Incorporation of these resistance genes in a wheat breeding programme is often hampered by the limitations of phenotypic selection on adult plants. Accurate identification of desirable germplasm can be restricted by environmentally dependent trait expression (presence and consistency of inoculum, plant development, temperature and humidity), large genotype by environment interactions (i.e. for grain yield), and availability of sufficient grain for destructive end use quality assessment. In addition, the time and resources required to reach a desired level of homozygosity in breeding populations restricts the rate of genetic gain.

The application of doubled haploid (DH) technology to wheat breeding has dramatically increased the speed at which wheat varieties can be developed. A function of DH technology is the loss of selection opportunities that would normally have occurred in early (F_2, F_3, F_4 etc) generations. However, direct genetic selection using molecular markers has been touted as an alternative to phenotypic selection, allowing more effective selection during the early stages of a DH breeding strategy (Howes et al. 1998; Radovanovic and Cloutier 2003). Integration of MAS within a traditional wheat breeding strategy may increase the accuracy and efficiency of selection but aside from targeted gene introgression through accelerated backcrossing, it is unlikely to improve the rate at which cultivars can be released. However, a combination of DH technology and MAS may provide breeders with the improvements in selection efficiency and varietal development rate that they seek (Howes et al. 1998).

Simulation studies have examined the potential role for MAS in breeding programmes (Hospital et al. 1997; Knapp 1998; Charmet et al. 1999; Moreau et al. 2000). These studies have shown that in some circumstances the adoption of MAS has the ability to improve selection efficiency over phenotypic selection alternatives. However, these studies considered the application of MAS on a theoretical basis in an attempt to characterise the 'global' improvements that MAS may provide to breeding. Although specific issues such as population size, gene action (i.e., additive or epistatic) and trait heritability were investigated, the authors did not consider the application of MAS in a specific germplasm pool, interacting with particular environments and selection regime. Other reports have detailed the successful application of MAS in pragmatic breeding programmes (Yu et al. 2000; Yousef and Juvik 2001; Jefferies et al. 2003; Zhou et al. 2003). Just one of these examples investigated selection for multiple traits (Yousef and Juvik 2001), and in each of the reports MAS was simply compared to a phenotypic alternative. Although the authors agreed that MAS can be an effective tool for plant breeding, they did not investigate the relative efficiencies of alternative MAS strategies to achieve the same outcome. In general, MAS was found to be more effective when the phenotypic alternative was either less cost efficient, not possible (e.g. due to insufficient quantities of grain, lack of disease pressure or recessive inheritance) or where the trait was of low heritability (Koebner and Summers 2003). While there are likely to be general advantages provided by MAS, the specific manner in which MAS is used within a breeding strategy may be just as important. Moreover, the authors of this paper believe that the application of MAS may be most prudently applied on a cross-by-cross basis.

The aim of this study was to evaluate particular methods of MAS when applied on a specific theoretical cross with a particular desired outcome, using computer simulation. Three possible phases of MAS were examined for a backcross one population; allele enrichment of BC_1F_1 DH donor plants, MAS haploid regenerates prior to chromosome doubling and marker-assisted recurrent parent selection before seed increase and grain yield selection. In this study, we examine how best to maximise genetic improvement whilst minimising costs through the application of molecular markers for a specific cross.

Materials and methods

Breeding scenario

AGT wheat cultivar, 'Stylet', gained particular attention from wheat producers in southern

Australia just prior to commercial release in 2000/01 due to its very high grain yield potential, rust resistance, cereal cyst nematode (CCN) resistance, and boron toxicity tolerance. However, during the ensuing 12 months, rust pathotypes developed with virulence to the three major resistance genes carried by 'Stylet' namely, *Lr37*, *Yr17* and *Sr38*. These separate events occurring over a short period of time, rendered the variety 'Stylet' susceptible to all three *Puccinia* species. Consequently, 'Stylet' was withdrawn from commercial release and wheat growers were unable to benefit from its improved grain yield and wide adaptation.

A restricted backcross defect elimination strategy was immediately commenced in 2002 in order to quickly produce a durably rust resistant version of 'Stylet'. 'Annuello', a moderate grain yield potential variety but believed to posses multiple adult plant rust resistance genes (R. Eastwood, personal communication) was backcrossed to 'Stylet'. In addition to the opportunity to improve 'Stylet's' rust resistance, the choice of 'Annuello' as the donor parent also provided the opportunity to enhance 'Stylet's' bread making qualities, largely through selection against the detrimental *GluA3e* HMW glutenin allele (Eagles et al. 2002).

Simulation design

Simulation software

The QUCIM module (Wang et al. 2003), of the genetic simulation software QU-GENE (Podlich and Cooper 1998), was used to conduct the simulation. Simulations were replicated through 30 models and 10 runs.

Genotype environment system

While genes controlling plant phenology, morphology (particularly plant height) and a large number of disease resistances have been well characterised, genes directly controlling or affecting grain yield are poorly understood in wheat. Therefore, for the purposes of this simulation, a simple genetic model was assumed. Twenty additive hypothetical grain yield *per se* genes were arranged on seven randomly assigned linkage groups with each linkage group also carrying a randomly positioned marker locus for genome selection. Known allelic differences between the parents for genes controlling major agronomic, physiological and disease resistance traits were included in the GE system. Gene locations, effects and linkage groups were based on published data (Bariana and McIntosh 1993; Jefferies et al. 2000; Ogbonnaya et al. 2001; Eagles et al. 2002; McIntosh et al. 2003; Suenaga et al. 2003; Williams et al. 2003). As such, independent segregation was assigned to the following loci; *GluA1*, *GluA3*, *GluB1*, *GluB3*, *GluD1*, *GluD3*, *Lr37*/*Yr17*/*Sr38*, *Lr34*/*Yr18*, *Lr46*/*Yr29*, *Lr24*/*Sr24*, *Rht1*, *Rht2*, *Rht8*, *Bo1*, *Cre1* and *Cre8*. Molecular marker loci linked to these genes were included in the GE system where appropriate (Table 1).

In order to accurately model AGT target wheat breeding region, four environment types (ET1, ET2, ET3 and ET4) were nominated to describe the major Australian wheat production zones, whilst a fifth environment (ET5) was included to represent a summer nursery within ET1. ET1, ET2, ET3, and ET4 correspond approximately to the southern, western, eastern and northern wheat growing regions of Australia, respectively. Each environment type was designated to represent the relative root and foliar disease reaction patterns, boron toxicity, and the grain yield level expected in each agrological region. Most pertinent to this simulation is the geographical dominance of particular rust races. For example, in environments ET1, ET3, ET4 and ET5, *Lr37* and *Sr38* were both fully effective and gave a resistant phenotype, whilst the third resistance gene at the VPM locus, *Yr17* (Bariana and McIntosh 1993), provided no protection against stripe rust infection. In contrast, for ET2 *Yr17* provided full protection against stripe rust whilst *Lr37* and *Sr38* were both ineffective against leaf and stem rust pathotypes common to this environment. Further complicating selection for rust resistance, only the disease nursery within ET4 was capable of providing effective selection for all three rusts types (although not all pathotypes). Similar differential geographic virulence effects for *Lr24* were incorporated into the model, necessitating the production of epistatic networks (EPNs) describing the gene interaction within each environment. EPNs for rust resistance were produced assuming that the effects of genes were cumulative to a maximum level of one (Table 2). Therefore, where two progeny, one possessing

Table 1. Recombination frequencies between target genes, and the molecular markers used for MAS.

Gene/Locus	Marker	Recombination frequency	Reference
Lr34/Yr18	*Xgwm295*	0.08[a]	Suenaga et al. (2003)
Lr46/Yr29	*Xgwm140*	0.30[a]	M. William (personal communication)
Lr24/Sr24	*Xgwm3*	0.12[a]	M. Pallota (personal communication)
Rht1	BF-MR1	0.00	Ellis et al. (2002)
Rht2	DF-MR2	0.00	Ellis et al. (2002)
Rht8	*Xgwm261*	0.01[a]	Korzun et al. (1998)
GluD1	P1 + P2	0.00	Ahmad (2000)
GluA3	*Xpsp2999*	0.00	Devos et al. (1995)

[a]Recombination values were selected to represent the approximate confidence interval of the QTLs.

Table 2. Assumed resistance levels conferred by the leaf, stem and stipe rust resistance genes from 'Stylet' and 'Annuello' within each of the simulated environments.

	Leaf rust		Stem rust		Stripe rust	
	ET1,3,4&5	ET2	ET1,3,4&5	ET2	ET1,3,4&5	ET2
Lr37/Yr17/Sr38	1	0	1	0	0	1
Lr34/Yr18	0.5	0.5	0	0	0.3	0.3
Lr46/Yr29	0.3	0.3	0	0	0.2	0.2
Lr24/Sr24	0	1	1	1	0	0

A score of one indicates complete resistance and zero, complete susceptibility.

Sr38 and the other possessing *Sr38* and *Sr24*, were grown in ET1,3 and 4 they would have equal levels of stem rust resistance. This genotype environment system accurately reflected the pathogen variability within Australia at the time of the simulation. For selection of grain yield, the target population of environments (TPE) was formed as a combination of ET1, ET2, ET3 and ET4.

Parental populations

For the purpose of this simulation, it was assumed that 200 BC_1F_1 seeds were produced from the cross 'Annuello/Stylet-c//Stylet-e', where 'Stylet'-c and 'Stylet'-e were selections from a 'Stylet' bulk heterogeneous at the *GluA3* locus (*GluA3c* and *GluA3e* segregating). The relative grain yield values for the two Stylet glutenin selections was unknown, and as such both were used in the construction of the population to ensure accurate representation of the 'Stylet' bulk. Within this simulation, 'Stylet-e' and 'Stylet-c' were considered identical except at the *GluA3* locus. Table 3 details the genetic assumptions concerning the genotypes of the two parents (R. Eastwood, personal communication; S. P. Jefferies, personal communication).

Breeding strategies

Four strategies, MAS0, MAS1, MAS2, MAS3 were investigated (Table 4). MAS0 was the control strategy employing phenotypic selection only. For MAS0, it was assumed that 4000 DH lines were created from 200 BC_1F_1 donors and then multiplied over summer. This location also acted as a disease nursery where leaf rust and stem rust resistance could be assessed. Surviving DH lines were then entered into preliminary grain yield, disease resistance and end use quality testing regimes (Figure 1). Two single replicate grain yield experiments were conducted within the main target location, ET1, while a single grain yield experiment was undertaken within both environments ET2 and ET3. Meanwhile, DH lines were also assessed at four disease nurseries (ET1, ET2, ET3 and ET4) to ensure selection for resistance to the various rust pathotypes and provide across site replication. Selection for end use quality was then performed on DH lines surviving grain yield and disease resistance assessment. Grain from each DH was milled, and maximum dough resistance (R_{max}) and dough extensibility (Ext) measured on the dough produced from the milled flour. No CCN resistance or boron tolerance assays were performed, as more than 50% of the lines

Table 3. Genetic characterisation of parents used to create the BC_1F_1 population under investigation.

Genes	Influence on yield?	Stylet	Annuello
Rust resistance genes			
Lr37/Yr17/Sr38		✔	
Lr34/Yr18			✔
Lr46/Yr29			✔
Lr24/Sr24			✔
Heterodena avena resistance genes			
Cre1			✔
Cre8 (+ Tolerance)	Yes	✔	
Quality genes			
GluA1		a	a
GluB1		c	b
GluD1		d	a
GluA3		e,c	b
GluB3		h	b
GluD3		c	b
Physiological genes			
Rht1	Yes		✔
Rht2	Yes	✔	
Rht8	Yes	✔	
Bo1	Yes	✔	
Hypothetical yield '*per se*' genes	Yes	13	7

Hypothetical grain yield genes are stated purely for simulation purpose.

Table 4. Description of the four selection strategies under genetic and economic investigation.

		MAS0	MAS1	MAS2	MAS3
BC1F1		**200**	**200**	**200**	**200**
MAS	*Lr34/Yr18*		✔	✔	✔
	Lr46/Yr29		✔	✔	✔
Haploids		**4000**	**4000**	**4000**	**4000**
MAS	*Lr34/Yr18*			✔	✔
	Lr24/Sr24			✔	✔
	Rht1			✔	✔
	Rht2			✔	✔
	Rht8			✔	✔
	GluD1			✔	✔
	GluA3			✔	✔
DH0		**4000**	**4000**	**720**	**720**
MAS	RP selection				✔
DH1		**4000**	**4000**	**720**	**511**
DN	ET5 (Lr, Sr, Ht)	✔	✔	✔	✔
DH2		**2574**	**2628**	**489**	**346**
YN	ET1	2	2	2	2
	ET2	1	1	1	1
	ET3	1	1	1	1
DN	ET1,2,3&4	✔	✔	✔	✔
Quality	Buhler Mill	32	48	26	18
DH3		**11**	**15**	**16**	**10**

Numbers in bold refer to the average number of lines entering each stage of the breeding programme for each strategy.
DN, Disease nursery; YN, Yield nursery.

produced by these breeding strategies should have carried both *Cre8* and *Bo1* (CCN resistance and boron toxicity tolerance, respectively). For the MAS1 strategy, selection followed that of MAS0 except that the 200 BC_1F_1 DH donor plants were pre-screened with markers linked to *Lr34/Yr18* and *Lr46/Yr29* to enrich the population for the favourable alleles at these loci. For MAS2, the MAS1 strategy was repeated but followed by marker screening of haploid regenerates to ensure that all haploids undergoing chromosome duplication were of a semi-dwarf phenotype, carried at least *Lr34/Yr29* or *Lr24/Sr24*, and had the potential to make high quality end products (through selection for *GluD1d* and *GluA3b/c*). Although within this cross *Sr24* would be required for adequate stem rust resistance in the target environment, known incomplete linkage between *Xgwm003* and *Lr24/Sr24* would restrict the effectiveness of selection. For MAS3, selection followed the regime outlined for MAS2 except that DHs were screened with a random set of markers in order to eliminate individuals that carried less than 30% of 'Stylet' (recurrent parent) genome.

Statistical analysis

Outputs from the simulation included final population means and variances for each of the traits, as well as the allele frequencies for each of the genes controlling these traits. These results were subjected to analysis of variance fitting strategy, and where appropriate, environment type as the explanatory factors, whilst using model and run as blocks. A stringent significance level of $p < 0.01$ was chosen to provide confidence against spurious positive associations.

Economic analysis

A spreadsheet-based assessment of the cost of field and laboratory-based selection at AGT was performed in preparation for an investigation into the relative costs of each of the four breeding strategies (Kuchel et al. unpublished data). DNA extraction was priced at $AUD 1.03 whilst each marker assay was estimated to cost $AUD 0.92, including PCR amplification, electrophoresis and allele scoring. Economic evaluations for phenotypic selection placed the cost of each grain yield plot at $AUD 6.42, whereas a disease nursery

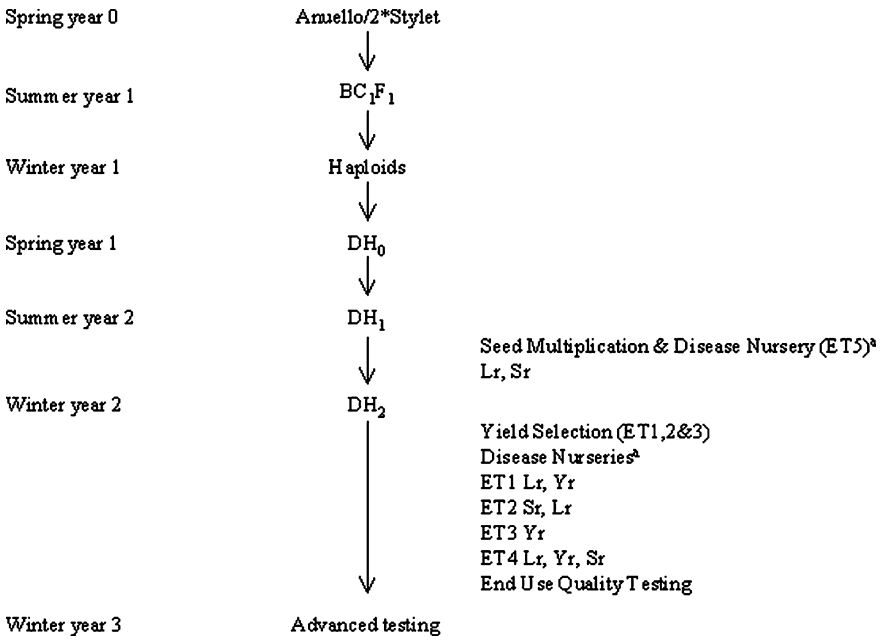

Figure 1. Diagrammatic representation of the basic phenotypic strategy (MAS0) being investigated for potential application of marker-assisted selection. [a]Within each disease nursery, selection could be performed for resistance against one or more of leaf rust (Lr), stem rust (Sr) or stripe rust (Yr).

observation row was estimated to cost $AUD 2.04 and out of season seed multiplication, $AUD 3.77. Other costs to be considered were DH production and end use quality assessment. Cost of doubled haploid production is estimated at $AUD 11.00 for haploid production plus $AUD 4.00 for colchicine treatment, whilst end use quality assessment was estimated to cost $AUD 150.00 per sample (S. P. Jefferies, personal communication). These prices were then used to calculate the overall costs of the four breeding strategies. Resource allocations were calculated, from the investment made on selection for each gene for each strategy. Resource allocation (G_{ij}) was calculated according to Equation 1. Where G_{ij} is the cost of selection of the ith gene for the jth strategy, S_k is the number of genes being exposed to selection during the kth selection stage and $C_{jk(i)}$ is the cost of the kth selection stage within the jth strategy.

$$G_{ij} = \sum_{k=1}^{n} \frac{1}{S_k} \times C_{jk(i)}, \qquad (1)$$

where $C_{jk(i)} = 0$ if the ith gene is not present for selection at the kth stage in the jth strategy. A gene was deemed to be exposed to selection when the trait it controlled was being assessed.

Results and discussion

Genetic progress

Grain yield

The analysis of variance for final population mean grain yield in the TPE showed that selection strategies MAS0, MAS1 and MAS2 did not significantly ($p < 0.01$) differ from one another, whilst MAS3 resulted in a population with a significantly higher mean grain yield. Although statistically significant, the magnitude of the grain yield advantage, less than one percent, was relatively small (Figure 2). Given that this simulation assumed one third of positive grain yield alleles would be provided by the donor parent, it was expected that the grain yield gain resulting from selection for recurrent parent genome could not be high, since it would inadvertently select against favourable yielding alleles from the donor parent. Moreover, selection for recurrent parent genome reduced the genetic variance available for selection in later stages. For instance, both MAS2 and

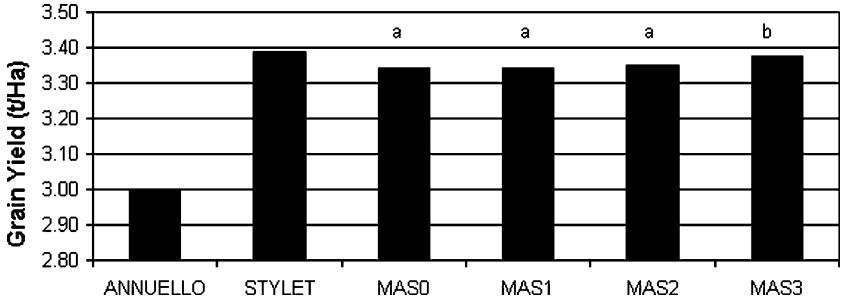

Figure 2. Effect of MAS strategy on genetic gain for mean grain yield. Means with a different letter significantly ($p < 0.01$) differ. Parents are included for comparison only.

MAS3 underwent the same selection events leading up to DH formation and consequently, at this point the two population's genetic variances for grain yield would be identical. However, the grain yield genetic variance after selection for recurrent parent genome in MAS3 (Vg_{yield} = 1.932 (t Ha^{-1})^2) was found to be significantly lower than that of MAS2 (Vg_{yield} = 2.152 (t Ha^{-1})^2). The general strategy employed a *restricted* backcross approach in order to allow the opportunity for transgressive segregation. Across all selection strategies, the final mean grain yield was appreciably higher than that of the donor parent, and no more than 1.5% below the recurrent parent. Therefore, the relative effectiveness of MAS2 and MAS3 is largely an economic rather than genetic consideration.

Rust resistance

Most critical to the outcome of this breeding strategy is the successful integration of multiple rust resistance genes, including adult plant resistance, into the 'Stylet' background, providing potentially durable and extensive protection from Australia's highly variable rust pathogenicities. In most environments (ET1, 3 and 4) *Lr37* and *Sr38* were effective against the common pathotypes, and as such, leaf rust and stem rust resistance reaction was of most interest in ET2 where *Lr37* and *Sr38* no longer provided protection. In contrast, *Yr17* was ineffective in ET1, ET3 and ET4, and consequently the results from these environments were of most importance as individuals with or without effective resistance genes underlying *Yr17* could be identified. There were no observed differences in the level of stem rust resistance between the final populations within ET2 (*Sr38* ineffective) indicating that neither marker-assisted selection at the BC_1F_1 nor haploid stages led to appreciable improvements in stem rust resistance (Figure 3). Conversely, the level of leaf rust resistance was significantly greater in MAS1 compared to MAS0, within ET2. Protection against leaf rust was further enhanced by selecting haploids carrying *Lr34* (MAS3 and MAS4). A very similar response was observed for the level of stripe rust resistance in ET1, ET3 and ET4. Selection for *Yr18* and *Yr29* at the BC_1F_1 stage, followed by selection for *Yr18* amongst the haploids significantly improved the level of stripe rust resistance. These changes in rust resistance were further investigated by analysis of the shifts in actual rust gene frequencies through selection (Figure 4). Selection at both the BC_1F_1 and haploid phases significantly increased the frequency of the primary gene target, *Lr34/Yr18*. Even though the linkage between the marker and gene for *Lr46/Yr29* was 'loose' (30 cM), a dramatic increase (25%, $p < 0.01$) in the frequency of the positive allele was observed following selection amongst the BC_1F_1 plants. As expected, selection using a marker closely linked to a gene (*Xgwm295–Lr34/Yr18*) was more effective than for 'loose' linkage relationships. However, the results for *Lr46/Yr29* show that even 'loose' marker–gene linkages can be very useful genetic tools for plant breeders. Manipulating the frequency of desirable *Lr24/Sr24* alleles with molecular markers appears to have been less successful (ca. MAS1 and MAS2). This is probably due to the phenotypic selection intensity for *Sr24* across all strategies. Only those lines carrying *Sr24* would meet the selection criteria imposed for ET2 where *Sr38* was designated as ineffective. This is not the case for *Lr34/Yr18* and *Lr46/Yr29* where, although both are desirable, only one of these genes was required to meet the selection criteria set for *Lr37* and *Yr17*

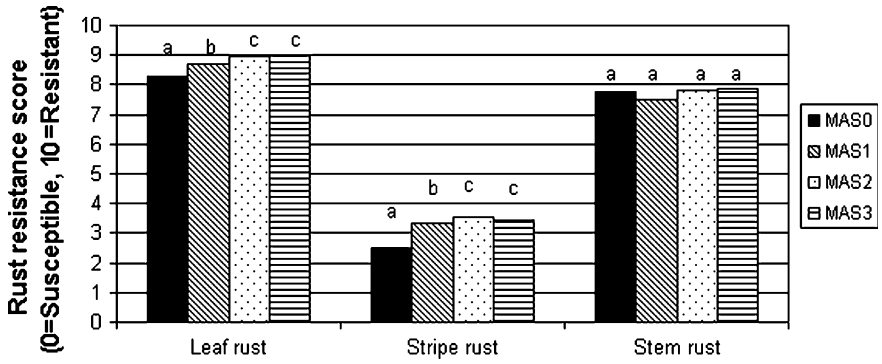

Figure 3. Effect of MAS strategy on rust resistance in NON-VPM effective environments. Means within each rust type with a different label are significantly different ($p < 0.01$).

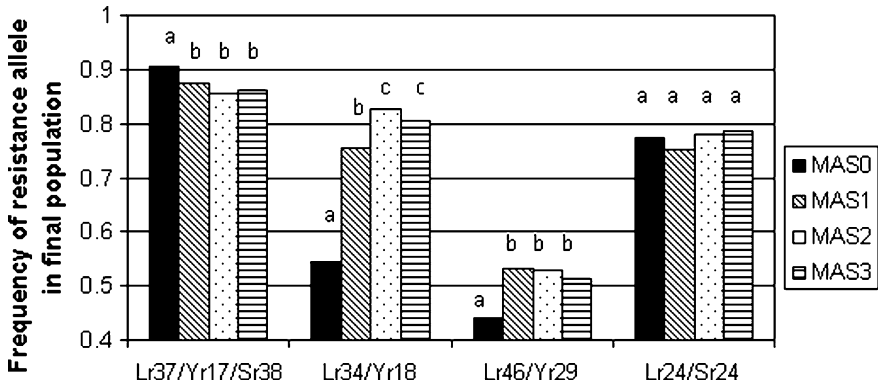

Figure 4. Rust gene frequencies arising from MAS strategies. Means for each rust gene with a different label are significantly different ($p < 0.01$).

ineffective environments. The relative effectiveness of the *Lr24*/*Sr24* MAS is therefore an economic rather than genetic issue study.

End use quality

In a similar manner to MAS for grain yield (recurrent parent selection), selection for end use quality traits was only performed on fixed lines, and was therefore ineffectual at manipulating the frequency of desirable haploids being produced. However, identification of those lines with inherently poor dough strength, assessed as the combination of both dough resistance and dough extensibility, prior to large scale grain yield and end use quality testing, reduced the resources spent on assessment of inferior quality lines in later stages (see economic results and discussion). The *GluA3e* subunit carried by 'Stylet' has been shown to confer very poor dough extensibility (Eagles et al. 2002), whilst the *GluD1a* allele carried by 'Annuello' has been reported to produce flour that makes dough with poor resistance (Payne et al. 1987; Eagles et al. 2002). Elimination of both these deleterious alleles in MAS2 and MAS3 considerably increased overall dough strength ($R_{max} \times$ Extensibility) when compared to MAS0 and MAS1 (Figure 5). This selection event was particularly successful, as it enabled a shift in the mean dough strength of the MAS2 and MAS3 populations *above* the dough strength of both parents.

Economic analysis

The application of MAS at the BC_1F_1 stage slightly increased the cost of the breeding strategy but improved the genetic gain made for leaf and stripe rust resistance (Table 5). Eliminating lines at the haploid stage significantly reduced the overall investment required to achieve the desired outcome. The lowest cost strategy, MAS2, proved to cost $AUD 65,000 less to achieve the same or

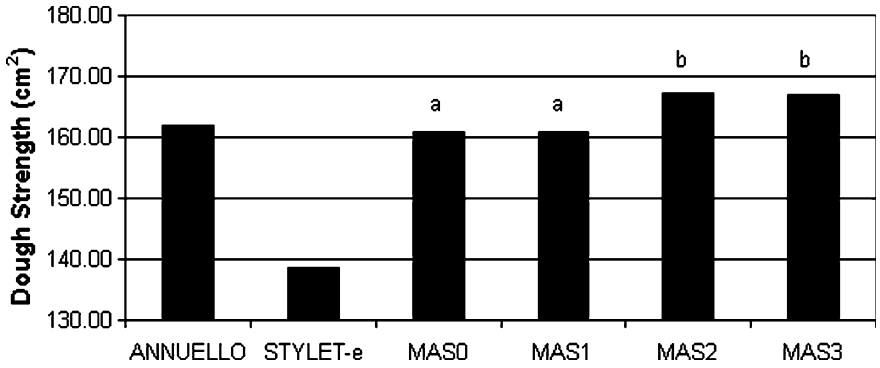

Figure 5. Response of dough strength in the Annuello/2*Stylet population to marker-assisted selection strategy type. The parents 'Stylet-e' and 'Anneullo' are presented for comparison only. Dough strength calculated as predicted R_{max} (cm) × Extensibility (cm). Dough strength from breeding strategies with a different label are significantly different ($p < 0.01$).

better outcome for each of the traits than the conventional alternative (Table 5). Although over $AUD 30,000 was spent on MAS for the MAS2 strategy, a total improvement in affordability was achieved through increased strategy efficiency. For MAS0, haploid lines not meeting the selection criteria for rust resistance and dough strength were subjected to chromosome doubling as there was no effective opportunity for phenotypic selection between haploid and DH phases. The resultant DHs were therefore included in summer seed multiplication, grain yield experiments, disease nurseries and end use quality testing. This result highlights the potential impact of MAS not just as an aid or replacement for phenotypic selection, but rather as a tool used to focus the allocation of resources in late generations to germplasm with a much greater probability of success. This shift in resource allocation is also evident on a gene-by-gene basis. An analysis of the expenditure on selection for rust resistance genes (Figure 6) reflects lower costs associated with MAS but also shows some interesting changes in the relative investment used in the selection of individual genes. For example, MAS for *Lr34/Yr18* at both BC_1F_1 and haploid stages as apposed the BC_1F_1 only (MAS2 vs. MAS1), reduced the overall cost of selection for all rust resistance genes but significantly increased the relative investment on the desirable *Lr34/Yr18* with respect to the other genes. Adoption of the MAS2 strategy also reduced the investment required for the selection of the less desirable *Lr37/Yr17/Sr38* resistance genes. These shifts in investment level demonstrate a successful integration of the aims of the breeding strategy (a reduction of the reliance on *Lr37/Yr17/Sr38*) and sound economic expenditure, both in terms of the strategy's resource allocation and its genetic outputs.

Much of this economic analysis is reliant on an accurate assessment of the resources required for

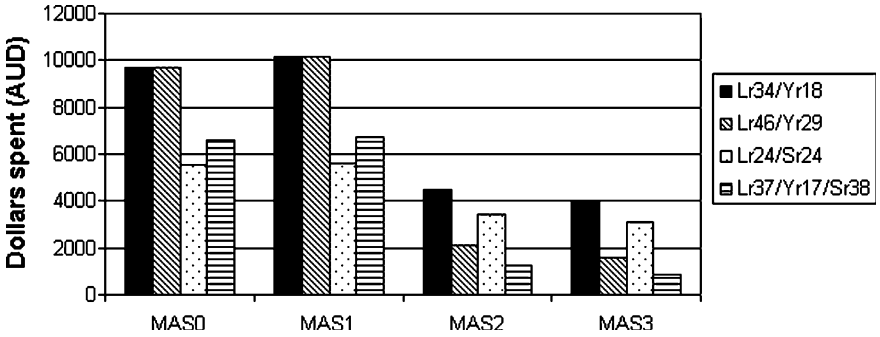

Figure 6. The cost of selection for the four rust genes under various marker-assisted selection strategies.

Table 5. Economic analysis of the four breeding strategies simulated for the Annuello/2*Stylet population.

	MAS0	MAS1	MAS2	MAS3
Molecular marker assays	$0	$574	$30,454	$59,016
Doubled haploid production	$60,000	$60,000	$46,880	$46,880
Summer seed increase	$15,080	$15,080	$2714	$1926
Grain yield trials	$66,100	$67,487	$12,558	$8885
Disease resistance nursery	$21,004	$21,444	$3990	$2823
End use quality tests	$4800	$7200	$3900	$2700
Total	$166,984	$171,786	$100,496	$122,232

Costs are presented in Australian dollars.

the different selection modes. Consequently, a sensitivity analysis was performed to determine the robustness of the conclusions drawn from the simulation. Final strategy costs were determined following alterations to the molecular marker and DNA extraction costs previously used for analysis. When costs were decreased by a half, the MAS2 strategy reduced in cost to $AUD 85,269.00, a drop of 15%. However, given the static cost of field analysis ($AUD 70,042.00 for MAS2) for any given strategy, further reductions in molecular marker assay costs do not significantly impact on overall strategy cost. In comparison, as molecular marker assay costs were increased their relative efficiency reduced. A 3.2-fold increase in the cost of DNA extraction and molecular marker assays resulted in MAS2 being equal in resource requirement to MAS0. This sensitivity analysis highlights two main points. First, the results drawn from this simulation are robust for the strategies being tested. It also suggests that although further research investment in marker technology will most likely lead to a reduction in the cost of MAS, maximising the benefits from these improvements is most likely to occur when breeding strategies are tailored to the changes in relative cost efficiency. This will require improved knowledge regarding key marker–trait associations as well as application of novel breeding systems.

Few studies have rigorously investigated the economic performance of MAS in breeding programmes. However, previous studies (Dreher et al. 2003; Morris et al. 2003) reporting the relative economic performance of a phenotypic and MAS strategy in maize indicated that the use of MAS could improve the rate of genetic gain, but was more expensive. In this case, Morris et al. (2003) considered a gene introgression scenario where markers were used simply for recurrent parent genome recovery. In another example, Moreau et al. (2000) concluded that the relative cost efficiency of MAS was largely reliant on the genetic foundation of the traits under selection, but also acknowledged that improvements in molecular marker technology could also make MAS more economically attractive. In comparison, this study has economically evaluated a specific and complicated marker-assisted breeding strategy utilising real gene locations and gene effects. The importance of this point should not be underestimated. In this study, the effectiveness of a specific disease resistance gene, the presence of additional genes affecting the same trait, and the timing of selection all had a significant impact on the relative effectiveness of MAS. Should another genotype environment system be considered, alternative conclusions would most likely be drawn. Although valuable themes may emerge from general studies, this work has shown that the economic success of MAS in a breeding programme will vary with the stage at which the markers are deployed and the number and value of genes selected.

In most of the previous simulations and analyses, MAS was considered simply within its role as an alternative to phenotypic selection. As such, many of the conclusions drawn fail to recognise the additional logistical rewards that MAS may provide when integrated with DH technology, the growing of multiple generations per year, and the advantages associated with a strategic rust selection strategy ensuring exposure to a full range of common races. The MAS strategies investigated in

this study gained many of their advantages from the timing of their application rather than from any benefit over a phenotypic selection alternative.

Conclusions

This genetic simulation and economic analysis has shown that MAS may not only provide improved genetic gain but also reduced cost. Many of the basic themes borne out by this simulation study could have been predicted through a good understanding of the genetic and environmental systems imposed in each breeding strategy. However, the *magnitude* of the genetic responses are less easily appreciated, particularly where genetic linkage, epistatic gene networks, and variable environments exist. In these cases, the use of computer simulation provides greater insights into the effectiveness of particular approaches. When combined with a detailed economic evaluation, simulations such as this can supply a breeder with confidence regarding their choice of breeding strategy. This study has also shown that in order to accurately assess the relative merits of different breeding strategies, both the genetic outputs and the economic requirements should be considered.

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