

# **“An economic assessment of the value of molecular markers in plant breeding programs”**

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## **Abstract**

The development of molecular markers allows plant breeders to identify desirable traits by analysing DNA rather than undertaking field trials or laboratory tests. This capacity provides a potential for a significant changes to occur within plant breeding programs. However, the value of marker-assisted selection varies with the trait assessed, the type of marker and the stage in the breeding program at which it is applied. In order to provide plant breeders with a basis for assessing the role of marker-assisted selection, the issues of which markers to apply and when to apply are analysed.

## **Key words**

plant breeding; molecular marker; economics; value

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## **1. Introduction**

### **1.1 Background**

The rapid increases in productivity in food production in recent decades have had improved plant varieties as a basic building block. Those varietal improvements have led to substantial gains in benefits globally (for example, see Evenson and Gollin 2003). At a more local level, there have been substantial gains in both wheat quality and yield in NSW in recent decades (Brennan and Bialowas 2001). These varietal improvements have come from the continued efforts of plant breeders to develop varieties that have the characteristics required by farmers to meet the production constraints in the different production environments.

Plant breeding programs have traditionally used “phenotypic selection”, combining direct measurement in the field, glasshouse or laboratory with statistical analysis, to allow breeders to search efficiently in large plant populations for progeny that exhibit desired traits (Dreher, *et al.* 2003). In recent decades, there have been significant technological improvements in both the screening methods available and in the statistical analysis that have allowed that process to become more efficient.

At the same time, the development of technologies associated with molecular markers has provided a tool that can replace some of this phenotypic selection and make the breeding process even more efficient. While the new technologies promise significant, often astounding, future benefits, there are several important practical considerations that need to be assessed before the most efficient role for molecular markers in plant breeding programs can be determined.

The aim in this paper is to provide an assessment of the potential role of molecular markers in wheat breeding programs, and to provide some empirical evidence of the relative costs and gains that can be achieved through the introduction of marker-assisted selection into wheat breeding programs.

In the next section, some further details of the marker technology are provided. The issues involved in determining the value of markers to breeders are then discussed. The costs of marker-assisted selection are assessed in the following section, and the costs of selected markers in the NSW Department of Primary Industries’ wheat breeding program at Wagga are estimated, as an illustration. The costs of the phenotypic evaluation that would be replaced by the markers are also estimated and compared to the costs of markers. In the final section, the findings are discussed, and the implications for breeders are considered.

### **1.2 Molecular markers**

The DNA-based molecular marker is a tool that can identify the presence of particular sections of DNA in an organism. A marker for a trait is defined when a particular segment of DNA is found to be correlated with the phenotypic expression (that is, an observable) characteristic. Some DNA markers can identify the presence of a particular gene (“perfect markers”). Others do not identify a specific gene, but rather a segment of DNA in the vicinity of the genes believed to be associated with the trait of interest (“flanking markers”). For these markers, there is uncertainty about the accuracy of a marker, since their identity is based on correlation, and those correlations are always less than complete.

Once a marker has been developed for a particular trait, breeders can use it to accelerate germplasm improvement efforts (Dreher *et al.* 2003) by:

- identifying favourable alleles in the genotype (that is, favourable segments of DNA in the breeding line);
- ensuring that the favourable alleles are carried through into the following generations through repeated cycles of selection; and
- identifying individual plants in large segregating populations that carry the desirable alleles.

Molecular markers provide a means of rapidly identifying the likely presence of a particular gene of interest (such as a gene for resistance to stem rust) in a breeding line. Marker-assisted selection can thus allow increases in the efficiency of identification and selection of lines with desirable characteristics, which otherwise can take several generations of evaluation. A large number of markers is currently available (with some already being used routinely), and many more are being developed. Key issues identified by Lindner (2004) are the intellectual property related to the molecular markers and the access by breeders to those markers.

There have been concerns expressed about the use of biotechnology to produce food products, but this aspect of the technology presents no element of environmental or food safety risk. The application of markers discussed here is for the exploitation of genetic variation that exists within the wheat plant or some closely related grass species. These technologies do not lead to alien genes being employed, or changes in genetic structure, but ultimately lead to similar varieties from a breeding program to those developed in conventional breeding programs, but may be able to lead to the development of those new varieties in a more efficient manner.

## **2. Value of Markers to Breeders**

### **2.1 Benefits from application of molecular markers**

The potential benefits that a breeding program can obtain from the use of molecular markers (Hassall & Associates 2002) are:

- (a) A significant reduction in the time to develop a new variety. The use of molecular markers can lead to more direct identification of the desirable traits than is possible through phenotypic selection, so that the time to develop a new variety (generally 10 years or more) can be reduced.
- (b) An increase in the overall rate of genetic gain in a breeding cycle. By allowing the more ready introduction of desirable traits in combination, the varieties produced using molecular markers can be more productive than those developed by conventional breeding methods.
- (c) The possibility of improving traits that were not possible to measure using traditional phenotypic screening. Some problems have been extremely difficult to assess using phenotypic screening, such as: (i) frost tolerance; (ii) pre-harvest sprouting; (iii) late maturity amylase in wheat; (iv) black point; (v) drought tolerance; (vi) tolerance to exotic pests and diseases such as Russian wheat aphid and Karnal bunt; and (vii) out-of-region breeding for constraints not applicable in the region where breeding is taking place.
- (d) Faster access to new genes to provide greater genetic diversity. By enabling screening for the presence of particular segments of DNA, molecular markers can allow the breeder to identify diversity at an early stage, which can be maintained through the breeding cycle.

While these benefits can potentially be obtained from molecular markers, to date their realisation in wheat breeding programs has been limited. The reasons for the limited success of practical applications of molecular markers to date include:

- (a) The relevance of the markers developed to selection criteria of the breeding program. For example, markers for cereal cyst nematode (CCN) are only likely to provide benefits for breeders developing varieties for the regions where CCN is a production constraint. Similarly, markers for aluminium tolerance are only likely to provide benefits for breeders developing varieties for the acid-soils regions where aluminium toxicity is a production constraint. Where a marker is widely applicable across different programs and product types, such as markers for protein glutenin composition in wheat, they have been quickly and widely adopted by breeders. Markers relating to specific productivity constraints may be quickly adopted in some programs while remaining of little interest to other breeders.
- (b) The extent to which the breeder can readily select for a characteristic phenotypically without a more costly use of markers (Morris *et al.* 2003). Markers for obvious physical traits such as plant height or grain colour can be of little practical interest to a breeder.
- (c) Even for some more complex traits, there can be a low-cost screening method available to a particular breeding program that is likely to prove less costly than the application of a molecular marker associated with that trait. For example, a very efficient system of field screening for *Septoria tritici* blotch has been in place at Wagga for many years (see section 4 below), and it is only likely to be replaced by a very low-cost marker.
- (d) The variation in the extent to which markers are applicable with different breeding populations. While a marker for a particular trait may be very useful to one breeder, it may have no value to another breeder who is using a different breeding population with different genetic variability for the relevant trait. This means that each marker must be validated for each genetic pool before it can be applied as a basis for selection within that pool. Such validation can be time-consuming and expensive for breeding programs, given its uncertain outcome, and can lead to a slow rate of adoption of new markers.
- (e) While some markers can be highly correlated with some characteristics identified by phenotypic screening, that correlation is never complete. Thus, breeders are faced with uncertainty as to the extent to which they can rely on the marker rather than the phenotypic screening. As a result, both systems are often run together in the initial stages of adoption of marker-assisted selection. This can mean an increase in costs during the transition stage between the traditional methods and the use of marker-assisted selection.
- (f) While markers for particular resistance to tolerance genes against pests or diseases can be valuable, the ever-evolving nature of such diseases means that other phenotypic evaluation will still be needed. Therefore, while a marker for a rust-resistant gene can be extremely valuable, relying solely on that marker for selection will make the varieties developed absolutely vulnerable to the break-down of the resistance of that gene, so a breeder would need to still undertake other phenotypic assessment of rust reactions in making selections. Thus, the possible cost savings from the use of that marker can be diluted considerably.
- (g) The extent to which molecular biologists are not working hand-in-glove with the breeders towards common goals of importance. It can be easier for a molecular biologist to work with more tractable problems, or to find simpler markers, than those that would prove really valuable to a breeder. While this was seen as a significant

problem in the earlier years of the development of markers, marker development is currently generally much better coordinated with the needs of breeders. The co-location of the molecular biology laboratory and the breeding program can be a key factor to assist in making those linkages stronger.

- (h) Reliable markers have not yet been developed for many characteristics of interest to breeders. For example, there is no “yield” marker, and the nature of the components of high yielding cultivars is such that such a marker may not be feasible, even in the long term.
- (i) The initial correlation (“linkage”) between the marker and the desirable trait may break down or erode over time. Therefore, the breeder may need to periodically conduct phenotypic assessment of the breeding lines to ensure that the marker-assisted selection remains relevant and useful. In the extreme case of a broken linkage, selection for the marker may result in selection *against* the desirable characteristic.

As a result, molecular markers have not replaced conventional evaluation and selection for many traits in the wheat breeding program at Wagga. Rather, markers are being introduced slowly, on a case-by-case basis, and have at times led to an increase in costs rather than a cost saving during the transition stage when validation is taking place or when the marker is first used (see below).

## 2.2 Value of a particular molecular marker

The value of a molecular marker to a particular breeding program depends on:

- costs of the marker implementation
- costs of alternative phenotypic selection regimes
- implications for the other operations of the breeding program.

The value of a molecular marker for a particular trait to an individual breeder will be determined by:

- the objectives of the breeding program
- the relevance of a particular trait in the key target region(s) for the breeding program
- the closeness of the linkage between the marker and the desirable trait
- the capacity to undertake phenotypic screening for the trait by conventional means, and
- the cost of phenotypic screening for the trait by other means.

As a result, the value of each molecular marker will be different for each breeder. Lindner (2004) assumes in his insightful exposition of the provision of essential plant breeding infrastructure that each molecular marker will be ranked in the same order of value by each breeder, but in practice that is unlikely to be the case, even if all breeders were targeting the same set of production environments. For example, some breeders would place different weighting on the production constraints in setting objectives for their program, so that markers would be given different values. This is magnified where breeders have different target environments. For example, breeders aiming to develop wheat varieties for South Australia are likely to put higher value on markers for boron tolerance and cereal cyst nematode (CCN) resistance than breeders targeting northern NSW, where neither trait is important in the productivity of varieties on farms.

Lindner (2004) also assumes in his analysis that each marker will cost the same to develop. Since the key components of marker development involve identification of the molecular marker and its relation to a genetic trait and validation of the linkage between the marker and the trait in relevant breeding populations, the costs are likely to be different. The costs of

development of populations for segregating different traits and the costs of the phenotypic evaluations to validate the marker can vary considerably between markers. For example, markers for rust resistance genes can be relatively less expensive to develop, given the availability of segregating populations, the knowledge of the genes themselves, and the ease of screening for such resistance. On the other hand, the costs are likely to be higher to develop and validate a marker for pre-harvest sprouting tolerance, where less is known of the genes involved, segregating populations are more difficult to develop, and the costs of assessing the degree of pre-harvest sprouting tolerance are high.

As a result, each marker is likely to have a different development cost, and its validation for different target breeding environments is also likely to vary. The time lags involved will also vary between markers. The value of those markers will in turn vary for breeding programs that have even subtle differences in objectives.

### **3. Costs of Marker-Assisted Selection**

#### **3.1 Cost components**

Once a marker has been developed and validated for a given environment and gene pool, it is ready for implementation into the breeding program, if it is advantageous. The costs of the implementation of a marker in a breeding program depend on a number of factors:

- (a) the cost and conditions of the intellectual property relating to that marker;
- (b) the costs of tissue collection and storage;
- (c) the method of extraction of DNA/protein for analysis;
- (d) the extent to which extracted DNA is used for a number of different markers;
- (e) the analytical method used;
- (e) the rate of throughput of the analytical system;
- (f) the degree to which the analysis is combined with that for other markers (“multiplexing”);
- (g) the ease of scoring the alleles after the analysis;
- (h) the costs of the labour required for the different operations (Dreher *et al.* 2003);
- (i) the capital cost of the laboratory equipment required;
- (j) the utilisation of the equipment required (that is, the overall throughput of the laboratory).

The extent to which there are economies of scale in the operations relating to different markers for different crops and situations is not explored in this paper. However, such a consideration, along with the extent of the capital equipment required for marker implementation on a routine scale, is important in determining the extent to which these facilities should be centrally located or whether they should be local components of each breeding program.

##### *3.1.1 Intellectual property issues*

The intellectual property embodied in a molecular marker is generally protected by patents or similar protective measures (Lindner 2004), and users are required to pay a licence fee. The form of the fee varies, but is commonly an annual licence fee. In some cases, a one-off fee entitles the breeder to use the marker in an unrestricted way for a number of years. The size of the fees also varies considerably between markers.

Where the breeding program develops its own markers, the fee does not apply, but the development costs would need to be accounted for. The extent to which the marker developed

is licensed to other users will have a large bearing on the true cost of the development to the program.

### *3.1.2 Costs of tissue collection and storage*

In plant breeding programs, the two forms of tissue generally used for DNA extraction are leaf and grain. In practice, in most cases, leaf samples are used as they are easier to work with and can be obtained within a few weeks of planting rather than waiting for the harvested grain samples. Where grain protein is being assessed, grain samples are required. The leaf samples must be obtained in the field or glasshouse, and frozen for storage until analysed. Special nurseries may be required to develop the plant material for extracting the DNA.

### *3.1.3 Method of extraction of DNA/protein for analysis*

Once the tissue samples have been collected, the next step in applying molecular markers is to extract the DNA from the plant tissue samples. In this operation, a small piece of leaf is placed into a cell (micro-tube) in the rack, along with two small ball-bearings. The rack is then shaken, and the action of the ball-bearings pulverises the leaf sample (Karakousis and Langridge 2003). A chemical solution is applied to that sample, and the DNA is extracted. Two different methods are commonly used for DNA extraction, using different chemicals (phenol-chloroform and sodium hydroxide). Where protein is extracted from grain samples, a specific protein extraction method is used. Each of these methods has different chemical and consumable costs.

### *3.1.4 Extent of usage of extracted DNA*

Since such minute quantities of DNA extract are needed for the analyses, once the DNA has been extracted for marker, it is possible to use the same DNA extract for a number of other markers. This can affect the marginal cost of the different analyses, making some markers economic where they would be more expensive if the full costs of DNA extraction had to be covered by that marker.

### *3.1.5 Analytical method used*

There is a wide range of analytical methods that can be used to analyse the DNA for the presence of markers. The specific options available for different markers are determined by the equipment available and the size of the DNA segment required for each of the markers, which can vary substantially. The rate of technological change is very high, and these techniques are evolving rapidly. The chemicals and other consumables differ between methods.

### *3.1.6 Rate of throughput of analytical system*

The rate of throughput of the system is a key component of the cost of the application of markers. If each sample were to be run individually, markers would be prohibitively expensive to operate. However, the modern systems are designed to work on arrays of samples, both for the extraction step and for the molecular analysis. The technology now in common usage relies on 96-well arrays undertaking 96 analyses simultaneously. These higher rates of throughput have led to rapid increases in the economic efficiency of the process.

### *3.1.7 Degree of multiplexing*

“Multiplexing” refers to the concurrent analyses for different markers. Where the markers are located in distinctly separate parts of the genome, analyses can be undertaken simultaneously. This means that one analytical process can enable determination to be made on more than one marker.

### 3.1.8 Ease of scoring alleles after the analysis

When the analysis has been completed, an operator is required to examine the output or allele scoring to determine the presence or absence of the appropriate DNA band associated with the marker. This is a task requiring considerable skill, and can take a significant amount of time.

### 3.1.9 Cost of labour

Since labour is a major component of the cost of the operations (see below), the cost of that labour and the availability of suitably trained operators is a significant factor in the economics of the different operations.

### 3.1.10 Capital cost of the laboratory equipment required

The application of molecular markers requires considerable specialised laboratory equipment to undertake the necessary analytical operations. The equipment varies with the different analytical techniques involved. In addition, because of the rapid rate of technological development in the area of biotechnology, the effective life of some of this equipment is shorter than would be expected otherwise. The equipment is likely to be obsolete or redundant long before the equipment physically wears out.

### 3.1.11 Utilisation of equipment required

Clearly, the extent to which expensive specialised laboratory equipment is utilised within the laboratory has a large impact on the costs associated with that equipment.

## 3.2 Empirical estimates of costs of marker application

As an illustration of the actual costs, empirical estimates are made for two markers currently used in the NSW Department of Primary Industries wheat breeding program at Wagga Wagga. One marker is associated with resistance to *Septoria tritici* blotch, a leaf disease of wheat, while the other is associated with a low level of polyphenoloxidase enzyme (PPO), a quality characteristic related to Prime Hard wheat.

The data used in the empirical analysis are as follows:

- Intellectual property fee is estimated at \$500 per year for the breeding program.
- The system uses a 96-well format for the sampling, extraction and analysis.
- Costs of tissue collection and storage: This operation is undertaken by a Technical Officer who obtains samples for four batches of 96 samples each day.
- The method used for DNA extraction is the phenol-chloroform method.
- Two analytical methods were costed, namely the agarose method and the polymerase chain reaction using the genotyper machine (PCR-CEQ) method. For the agarose analysis, one Technical Officer can analyse four 96-sample batches per day, while for the PCR-CEQ analysis only three batches per day are analysed. The chemicals and other consumables are estimated as \$0.15 sample for the agarose analysis and \$3.68 per sample for the PCR-CEQ analysis.
- The scoring of the results of the analysis takes 0.5 hours per batch by the molecular biologist for the agarose analysis, while for the PCR-CEQ analysis it requires 1.0 hour of the molecular biologist and 0.25 hours of the Technical Officer per batch.
- The labour costs are based on salaries and on-costs in the NSW Department of Primary Industries.
- The estimated total new replacement cost of the equipment in the marker laboratory in Wagga Wagga is approximately \$818,000, although only a part of this equipment is used for marker-assisted selection, as some elements are related to research and the

development of markers. Annual depreciation and interest charges of approximately \$63,000 need to be met from the marker applications analysed. The capital costs per marker vary with the number of each analysis undertaken with each piece of equipment and the costs of the equipment required. The costs per sample range from \$1.14 per sample for agarose analysis to \$5.21 per sample for PCR-CEQ analysis.

- No multiplexing is used for these markers.

On this basis, preliminary estimates of the cost components of the two methods are shown in Table 1. The agarose analysis costs \$7.97 per sample, while the PCR-CEQ analysis costs \$16.33 per sample, on the basis of current throughput levels at Wagga. Labour, operating and capital costs form relatively similar shares of the costs of the agarose analysis, while for the PCR-CEQ analysis the capital costs dominate (and therefore have a greater capacity to fall if throughput levels were increased). However, each component of costs is higher for PCR-CEQ than for the agarose analysis.

**Table 1: Estimated Costs of Methods for Marker Application**

	<b>Tissue collection and DNA extraction</b>	<b>DNA analysis and scoring</b>	<b>Total</b>
<b>Analysis (Agarose)</b>			
Labour	\$1.81	\$1.27	\$3.08
Operating	\$1.35	\$1.11	\$2.46
Capital costs	\$1.28	\$1.14	\$2.42
<b>Total costs</b>	<b>\$4.44</b>	<b>\$3.52</b>	<b>\$7.97</b>
<b>Analysis (PCR-CEQ)</b>			
Labour	\$1.81	\$2.07	\$3.88
Consumables	\$1.35	\$4.61	\$5.96
Capital costs	\$1.28	\$5.21	\$6.49
<b>Total costs</b>	<b>\$4.44</b>	<b>\$11.89</b>	<b>\$16.33</b>

These costs represent the costs of the application of the methods themselves, and do not include all of the costs of the application of markers. The licence and IP costs, data management costs of the operations and the costs of growing the plant in the glasshouse for the DNA sampling need to be included. The costs of two alternative forms of analysis for applying the marker for *Septoria tritici* blotch are estimated in Table 2. The preliminary estimates of the total costs per sample of using this marker are \$11.12 for the agarose analysis and \$19.48 for the PCR-CEQ analysis.

The estimated costs of applying the marker for PPO using the same analyses are the same as shown in Table 2, given the same licence fee.

**Table 2: Estimated Costs of Applying Marker for Septoria Tritici Blotch**

	<b>Analysis using Agarose method</b>	<b>Analysis using PCR-CEQ method</b>
Licence and IP costs	\$0.22	\$0.22
Data management	\$0.47	\$0.47
Growth of leaf samples	\$2.46	\$2.46
- <i>sub-total</i>	\$3.15	\$3.15
Tissue collection	\$0.91	\$0.91
DNA extraction	\$3.54	\$3.54
Analysis	\$3.16	\$11.03
Scoring of alleles	\$0.37	\$0.86
- <i>sub-total</i>	\$7.97	\$16.33
<b>Total costs</b>	<b>\$11.12</b>	<b>\$19.48</b>

## 4. Costs of Phenotypic Evaluation Replaced by Molecular Markers

### 4.1 Role of phenotypic selection methods

The alternative to the use of molecular markers in a breeding program is to measure the traits directly in the field, glasshouse or laboratory (“phenotypic assessment”) for use as a basis for selection of lines to retain in the program. For some characteristics such as plant height, this characteristic is readily assessed in the field by a low-skilled operator at a fast rate, and has a low cost. Other assessments need elaborate field sampling and laboratory testing to determine the expression of that characteristic in a breeding population. Other characteristics such as Septoria tritici blotch resistance can be assessed in the field by growing the nursery in conditions to ensure an epidemic, and then scoring the disease expression in the field, a skilled task that can be done rapidly. Root diseases such as cereal cyst nematode and crown rot are much more difficult (and expensive) to score in the field. Glasshouse screening can be used for several characteristics, but can be relatively expensive. A broad characterisation of phenotypic selection for some selected traits is shown in Table 3.

**Table 3: Characterisation of Phenotypic Selection for Some Selected Traits**

<b>Trait</b>	<b>Where measured</b>	<b>Operator skill</b>	<b>Speed</b>	<b>Expected cost</b>
Plant height	Field observation	Low	High	Very low
Septoria tritici blotch resistance	Field nursery	Medium-high	High	Low
Septoria tritici blotch resistance	Glasshouse	Medium-high	Medium	Medium
Cereal cyst nematode resistance	Field & laboratory	Medium-high	Low	Very high
Crown rot resistance	Field & laboratory	Medium-high	Low	High
Leaf rust resistance	Field nursery	Medium-high	High	Low-medium
Dough glutenins in wheat	Laboratory	Medium-high	Medium	High
Wheat quality (PPO)	Laboratory	Medium	High	Low
Karnal bunt resistance	Overseas (field)	Medium	Very low	High

In this study, the costs of two types of phenotypic assessment were evaluated in detail: (a) *Septoria tritici* blotch; and (b) wheat quality, measured as polyphenoloxidase (PPO). In each case, the costs were estimated from the operations in place for the wheat breeding program at the Wagga Wagga Agricultural Institute.

#### 4.2 Empirical analysis of phenotypic evaluation of *Septoria tritici* blotch

For *Septoria tritici* blotch, two forms of evaluation are used, namely field nursery testing and glasshouse screening. The field evaluation takes place under irrigation (centre-pivot) to provide the moist humid conditions favouring a *Septoria* epidemic. The lines are then scored for the disease during the season by experienced operators, but the nursery is not taken through to harvest. The glasshouse operations are much more labour-intensive. Plants are grown in individual pots, and the inoculum spread to ensure infection. Again the plants are scored for the disease by experienced operators. Because the capacity of the glasshouse is much less than the field (only 300 lines can be evaluated at one time), repeated cycles throughout the season are required in the glasshouse.

The costs differ widely between the two evaluations (Table 4). Field testing costs \$2.44 per line evaluated, while in the glasshouse the costs are \$15.22 per line. In each case, labour is the overwhelmingly major component of the costs, as found by Dreher *et al.* (2003) for maize plantings at CIMMYT. While some lines are evaluated only in the field, and some only in the glasshouse, lines at the later stages of the program are evaluated in both field and glasshouse, which costs a total of \$17.66 per sample.

**Table 4: Phenotypic Evaluation of *Septoria Tritici* Blotch at Wagga Wagga**

	Field testing	Glasshouse	Total
Labour	\$2.14	\$14.54	\$16.68
Operating	\$0.14	\$0.15	\$0.28
Capital cost	\$0.16	\$0.53	\$0.69
<b>Total costs</b>	<b>\$2.44</b>	<b>\$15.22</b>	<b>\$17.66</b>

#### 4.2 Empirical analysis of phenotypic evaluation of PPO

For PPO, the laboratory process is a relatively simple one. A Technical Assistant can complete 504 samples per week, and consumable costs are negligible. Thus, operating costs (including labour) are estimated at \$4.96 per line. The equipment needed has annual capital costs of \$3,411, but is shared across a number of different analyses. The capital costs amount to \$2.21 per sample, or \$2.21 per line assessed for PPO. Thus, the total costs of phenotypic assessment of PPO are \$7.17 per line (Table 5).

**Table 5: Costs of Phenotypic Evaluation of PPO**

	Costs per line
Labour	\$4.88
Consumables	\$0.08
Capital cost	\$2.21
<b>Total costs</b>	<b>\$7.17</b>

## 5. Discussion and Implications

### 5.1 Cost comparison of marker-assisted selection and phenotypic selection

The broad parameters for the successful implementation of marker-assisted selection are:

- Marker implementation costs
- Accuracy of marker
- Relevance of trait to target environment
- Capacity for alternative screening
- Cost for alternative screening

In the case of *Septoria tritici* blotch, markers cost \$11.12 per sample for agarose analysis and \$19.48 for PCR-CEQ analysis. Clearly, the former is preferable from a cost point of view. The phenotypic evaluation for *Septoria* costs \$17.66 per line where both field and glasshouse screening is undertaken. Thus, if the agarose-based marker is used, and replaces both field and glasshouse screening, then there can be considerable cost savings. Even replacing only the glasshouse component with the agarose-based marker would also lead to savings. However, for those lines currently only tested in the field screening (the majority of lines), the costs would increase considerably with the application of markers. At present, 7,000 lines are only tested in the field, 1000 are only tested in the glasshouse, and 3,250 are tested in both, which costs \$90,000 per year. If this was replaced by agarose-based marker analysis, the total costs would be \$125,000 per year (an increase of 40%); if replaced by the PCR-CEQ analysis, the costs would more than double to \$219,000 per year.

For PPO, the phenotypic evaluation costs \$7.17 per line, compared to \$11.12 for the agarose-based marker. As a result, replacing the laboratory testing with the marker for PPO would result in a 55% cost increase. If the testing were replaced with a PCR-CEQ -based marker, the costs would almost treble the current cost.

### 5.2 Discussion of the results

These comparisons are only part of the picture. Where such marker analyses could utilise shared DNA samples, the extraction costs would be reduced, and hence the total costs. For example, if four analyses could be carried out from the one DNA extract, the marker costs would fall by over \$5 per sample, or 53% for the agarose analysis and 27% for the PCR-CEQ analysis. Similarly, if the markers could be applied in a multiplexing system, the analysis costs could be reduced, further reducing the total costs of the markers. With three markers multiplexed (*ie* three analysed simultaneously), costs would fall by a further 50%. Thus, the markers would be able to lead to significant cost reductions, where both multiplexing and shared DNA samples were employed.

Those gains would, however, only result where the markers were able to fully replace the phenotypic selection. It seems unlikely that such complete replacement will occur in the near future, at least for the *Septoria* marker, so that those cost savings may not be able to be realised for some time. Only when the markers are sufficiently robust to enable the breeders to replace phenotypic selection altogether for that characteristic, along with an efficient system marker application, are the long-promised cost savings of markers likely to be captured.

The marker-assisted selection and conventional phenotypic selection may not be perfect substitutes (Morris *et al.* 2003). In some cases, markers can provide information on allelic composition that is not available through phenotypic assessment. The differences in time for results from the marker-assisted selection and conventional phenotypic selection for some

characteristics can mean that the former provides effectively a different output, in terms of its usefulness in selection. On the other hand, field observation for one trait can lead to valuable information about other traits, so that the information from one approach is not directly comparable to that of the other.

The two examples discussed cannot be used to make broad generalisations about other markers. As we have seen above, both the costs and the value of markers varies widely for each marker in each breeding program. As a result, there will be some markers that can lead to significant cost savings for some breeders, while there will be others that will not lead to cost savings. In the interim, while breeders are assessing the relevance and the value of the wide range of new markers that are available, it can be necessary to run both the phenotypic selection and the markers at the same time, and that leads to increases in costs rather than reductions.

In addition, the costs of both markers and phenotypic evaluation vary with the throughput of the systems used, because of economies of scale. For example, if considerably larger numbers of samples were put through either system, the costs per line evaluated are likely to decline. Thus, the PCR-CEQ costs could fall sharply if greater utilisation were made of the genotyper machine used, as the high capital costs per sample would be markedly reduced. Dreher *et al.* (2003) show the importance of throughput in unit costs, and similar analysis needs to be undertaken to make similar comparisons in this context.

There are also significant developments taking place in the marker technology, such as genome-wide analysis, whereby potentially hundreds of markers can be applied in a single analysis. These systems offer additional applications to breeders other than simple trait-marker associations. However, in the case of trait-marker associations, they offer a potential to alter the costs for each marker, and may enable the relative costs to be reduced considerably. However, such markers need to be relevant to the particular breeding program. If the system only allows breeders to reduce their phenotypic selection for a small number of characteristics, because so many of those applied relate to characteristics not relevant to that program, then there may still be no cost savings.

Where there are no or only minor cost savings from the use of markers, the choice of selection technology for the breeder to use is likely to depend on factors other than the cost per sample, such as (Dreher *et al.* 2003)

The question of when to apply markers in the breeding program is a critically important one for the breeders, as a strategic use of markers in the early generations can have considerable down-stream consequences for later in the selection process. The populations the breeder is working with can be genetically enriched by the strategic use of selected markers at an early stage. However, no attempt has been made to address that complex issue in this paper.

In conclusion, any statements about the improved efficiencies in terms of breeding costs as a result of the use of molecular markers needs to recognise the difficulties in generalising about markers. They can, indeed, lead to cost savings in some situations, but they can also lead to cost increases in other cases. The combination of valuable characteristic, low-cost analysis, reliable marker and higher costs of phenotypic selection is the most likely to lead to valuable outcomes for the breeders.

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