Cost analysis of molecular techniques for marker assisted selection within a barley breeding program

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Abstract

Marker assisted selection (MAS) has become a valuable asset to plant breeders as it provides an alternative strategy to introgress valuable alleles into breeding material. As part of its integration into mainstream breeding, finding reliable low cost, high throughput screening techniques is an important aspect of most implementation programs. With recent advancements in molecular marker techniques and the growing availability of commercial genotyping services, there are now a number of options available to breeders for the implementation of MAS.

This paper explores the value of three genotyping methods for marker assisted breeding in barley, namely: the traditional approach using conventional PCR and ethidium bromide stained polyacyrlamide gels, a new technology employing multiplex PCR and fluorescent-based marker detection on an automated DNA fragment analyser, and outsourcing to a commercial service provider. A doubled haploid population screened using these methods are compared in relation to the cost, speed, convenience and reliability of each approach.

Key Words

high-throughput genotyping, marker assisted selection, multiplex-ready markers, cost analysis

Introduction

Marker assisted selection (MAS) provides the opportunity to implement a range of unique techniques to improve selection efficiency in cereal breeding. Traditionally marker screening technologies have been expensive to implement, due to infrastructure requirements and cost and time required to perform marker assays. However, recent advances in molecular marker technologies, especially PCR and DNA extraction methods, have significantly increased the speed with which marker assays can be performed at reduced costs. These technological advances now provide the opportunity for cereal breeding programs to more vigorously adopt MAS as a tool to increase the amount of genetic gain that can be captured in each breeding cycle. In addition, a number of commercial genotyping service providers have emerged to provide further opportunities for MAS in cereal breeding. Commercial genotyping services are likely to be utilized by breeding programs lacking the infrastructure required to perform certain types of marker assays, and having genotyping requirements that are beyond the current screening capacity of their laboratories.

The University of Adelaide Barley Breeding program has been implementing MAS since 1995, and currently screens approximately 20,000 genetic loci per year. This throughput capacity has been achieved through the use of high-throughput DNA extraction methods and PCR-based markers. Until recently, marker screening has been performed using standard PCR assays and ethidium bromide stained, non-denaturing polyacyrlamide gels, as described by Karakousis et al (3). This approach has proven robust, but has the inherent disadvantage that it allows the screening of only a single marker at a time and is labour intensive. As a consequence, our laboratory has essentially reached its current throughput capacity without significant investment in infrastructure and personal. More recently, our capacity to achieve a higher level of genotyping throughput was made possible by the development of a new technology platform, called multiplex-ready markers (2). This technology couples multiplex PCR marker amplification with fluorescent-based detection on an automated DNA fragment analyser, and allows on average 20 markers to be screened in a single assay. With current access to an ABI3730 through a commercial service provider, this technology not only has the potential to significantly increase marker screening throughput, but also to reduce genotyping costs.

The objective of this paper is to explore the value of the three technology platforms that are currently available to our barley breeding program from an economic and application prospective: namely, our current approach based on standard PCR and ethidium bromide stained, non-denaturing polyacrylamide gels, multiplex-ready markers, and outsourcing to a commercial service provider. A doubled haploid population was used to compare the costs and relative advantages of the three technologies.

Methods

A doubled haploid population consisting of 309 individuals and two parents were screened with six trait-linked markers. For conventional and multiplex-ready PCR assays performed in our laboratory, DNA was extracted from each line using a freeze-dried method (1).

Conventional marker assays

The doubled haploid population and parental lines were screened a single marker at a time using conventional PCR and ethidium bromide stained, non-denaturing polyacrylamide gels, as described by Karakousis et al (2).

Multiplex-ready marker assays

The expected allele sizes for the six SSRs in the doubled haploid population allowed three markers to be co-separated per dye detection channel on an ABI3730. Using 5-dye detection, two DNA samples could be assayed for all six markers in a single ABI3730 capillary. Two 3-plex PCR assays were performed for each DNA sample, as described by Hayden et al. (2005). The PCR products were then post-PCR pooled and desalted by ultrafiltration in preparation for electrophoretic analysis on the ABI3730 (2). ABI3730 separation was performed by a commercial service provider. Marker data analysis was performed within our laboratory using GeneMapper v3.7 software.

Commercial genotyping service provider

Harvested leaf material for the parental and doubled haploid lines was sent directly to the commercial service provider for DNA extraction using a propriety method. Genetic analysis of the six markers was performed on an ABI3730, and analysed data for each doubled haploid line was supplied to our laboratory in the form of a Microsoft Excel report.

Cost Analysis

Calculations for the cost of marker genotyping performed using conventional and multiplex-ready assays are based solely on the actual cost of consumables, labour and equipment depreciation due to wear and tear. This approach was adopted in view that other operational overheads, such as institutional and building costs, are fixed costs that the barley breeding program would continue to pay, irrespective of the marker genotyping platform used. Table 1 shows a breakdown of the cost to perform each step required to genetically analyse the six markers on a single DNA sample. The costs presented in Table 1 include DNA extraction, PCR assays, product separation, and marker scoring. The cost of growing and harvesting leaf material for each doubled haploid line was not included, as this step was common to the three marker screening methods tested.

Table 1. Cost of screening the six trait-linked SSRs on each doubled haploid line using conventional and multiplex-ready PCR

		Conventional Assay **Cost per DH line**	Multiplex-ready Assay **Cost per DH line**
Reagents / Consumables	DNA extraction	$0.12	$0.12
	PCR	$0.96	$0.20
	Gel separation & analysis	$0.54	$1.20
Labor*	DNA extraction	$0.59	$0.59
	PCR	$1.33	$0.44
	Gel separation & analysis	$1.78	$0.44
Equipment replacement, wear and tear	DNA extraction	$0.38	$0.38
	PCR	$0.72	$0.24
	Gel separation & analysis	$0.17	n.a.
	Total	**$6.59**	**$3.61**

n.a.; indicates no associated cost because gel separation was performed by a commercial genotyping service provider.
*based on a technician salary of $40,000 per annum plus 30% on-costs

The cost of screening the six-trait linked markers per doubled haploid line using the commercial genotyping service provider was calculated as the total amount charged divided by the lines assayed.

Results and Discussion

The purpose of this paper was to compare three marker genotyping platforms that are currently available to our barley breeding program from an economic and application prospective. The three technologies compared were our current in-house approach based on conventional PCR and ethidium bromide stained polyacrylamide gels, the recently developed multiplex-ready marker technology (2) which involves multiplex PCR and fluorescent-based marker detection on an automated DNA fragment analyser, and genotyping performed by a commercial service provider.

Comparisons of the genotypic data generated by the three approaches showed a high (95%) concordance, indicating that all technology platforms were capable of delivering high quality data. Inspection of the genotypic data revealed that failed PCR reactions were the main cause of discrepancies between the three methods, but no approach showed a higher rate of PCR failure than the other (data not shown).

In terms of genotyping costs, the multiplex-ready marker technology was the cheapest to perform. Comparison of the overall costs incurred for genotyping the population revealed that our conventional approach and the use of a commercial service provider were both two and three times more expensive than the multiplex-ready marker technology respectively (Table 2). Comparison of the multiplex-ready marker system and the conventional approach revealed little difference in the cost of consumables. Rather, the higher cost incurred with the conventional protocol was primarily a consequence of the additional labour that was required to perform gel separation and marker scoring. In contrast, gel separation for the multiplex ready technology was performed on the ABI3730 where marker scoring was essentially automated using the GeneMapper software and in-house scripts that translate GeneMapper data into Microsoft Excel reports. Also contributing to the higher labour cost for the conventional protocol was the requirement to perform a total of six PCR assays for each DNA sample, compared to only two 3-plex PCR reactions for the multiplex-ready technology. The larger number of PCR assays performed and separation of PCR products on polyacyrlamide gels also contributed to a higher cost for equipment depreciation, and wear and tear. These results showed that the multiplex-ready marker technology would be the cheapest genotyping platform to implement in our breeding program.

Table 2. Comparison of costs relating to the genotyping of 311 barley lines using three methods.

		Conventional assay			Multiplex-ready assay			Commercial service provider
		per sample	total cost	% of cost	per sample	total cost	% of cost	total cost
Reagents & consumables	DNA extraction	$0.12	$37.32	1.9%	$0.12	$37.32	3.5%	
	PCR	$0.96	$298.56	15.1%	$0.20	$62.20	5.7%	
	Gel sep. & analysis	$0.54	$167.94	8.5%	$1.20	$373.20	34.6%	
	Cost per sample	$1.62			$1.52			
	TOTAL COST		$503.82			$472.72		
Labor	DNA extraction	$0.59	$183.49	9.3%	$0.59	$183.49	17.0%	
	PCR	$1.33	$413.63	20.9%	$0.44	$136.84	12.7%	
	Gel sep. & analysis	$1.78	$553.58	28.0%	$0.44	$136.84	8.6%	
	Total time taken	$3.70			$1.33			
	TOTAL COST		$1150.70			$457.17		
Equipment replacement wear & tear	DNA extraction	$0.38	$118.18	6.0%	$0.38	$118.18	11.0%	
	PCR	$0.72	$223.92	11.3%	$0.24	$74.64	6.9%	
	Gel sep. & analysis	$0.17	$52.87	2.6%	n.a.			
	Cost per sample	$1.27			$0.62			$11.98
	TOTAL COST		$377			$193		
	OVERALL COST		**$2,032**			**$1123**		**$3,726**
	Cost per genotype		**$1.09**			**$0.60**		**$1.99**

From a technological standpoint, fluorescent-based genotyping on an automated DNA fragment analyser, such as the ABI3730, provides several distinct advantages for high-throughput marker genotyping. The resolution achieved on a DNA sequencer allows two base pair allele size differences to be readily discriminated. In contrast, the resolution achieved on ethidium bromide stained, non-denaturing polyacrylamide gels are usually limited to about a four base pair difference. The capacity of automated DNA fragment analysers to achieve high resolution allele discrimination is particular important in breeding programs, where allele size differences are often small and heterozygosity is common. The latter is particularly important in top-cross breeding strategies and F_2 populations. Fluorescent-based genotyping also facilitates the co-separation of several markers in a single capillary (i.e. gel lane) and enables semi to fully automated marker scoring, thereby significantly increasing genotyping throughput. In contrast, the ethidium bromide stained, polyacrylamide gels used in our conventional approach allow only a single marker to be separated at a time due to heteroduplex formation. In this context, the multiplex-ready marker technology and genotyping provided by commercial service providers are expected to find greater utility in breeding programs, as breeding methodologies attempt to use larger numbers of markers to select and combine the most favourable regions of the genome.

Conclusion

In summary, we believe that the three genotyping methods have inherent advantages and disadvantages for cereal breeding programs. Our conventional approach is a robust system that currently allows up to 20,000 genotypes to be scored per year. However, it is unlikely that a significant increase in throughput could be achieved due to the time and labour required for gel separation and analysis. In contrast, fluorescent-based genotyping which is obtainable either by using the multiplex-ready marker technology or by outsourcing to a commercial service provider would allow a significant increase in genotyping throughput to be achieved. With the increasing use of MAS in our barley breeding program, access to a fluorescent-based genotyping technology will be paramount. In addition, these genotyping approaches vastly improve allele resolution and the ability to score the heterozygous allelic state. The results of this study indicate that the multiplex-ready marker technology would allow us to achieve about a two-fold increase in genotyping throughput per annum based on our current operating budget (Table 2). Outsourcing to a commercial service provider would reduce our level of throughput per annum without additional funding, due to the higher 2-fold cost per genotype (Table 2). Overall, it is likely that the breeding program will continue to use our conventional approach, as well as adopting the multiplex-ready marker technology. The advantage of our conventional approach lies in its suitability to readily perform small-scale impromptu screening of breeding lines and validation

experiments. Commercial genotyping services may also prove useful for during peak period when sample numbers are high and additional staff are not available.

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