The challenges of integrating new technologies into a wheat breeding programme

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ABSTRACT
William Farrer, who is considered the father of wheat breeding in Australia, recognised the importance of adopting the latest in genetic and biological understandings to improve the efficiency and effectiveness of his breeding programme. Since their inception, breeding programmes around the world have used advances in science and technology to improve rates of genetic gain. Recently, the field of molecular biology has offered breeders the tools to select elite individuals based on their genotype rather than, or in addition to, their phenotype. But it is one thing to have the tools, and quite another to integrate them effectively within an applied breeding programme. This challenge is not just limited to molecular biology. There are constant developments in physiology, biometry, computer simulation, engineering, end-use quality assessment and robotics, amongst others, that need to be continually assessed to identify which will provide the greatest return in terms of rates of genetic gain and cost efficiency. With a focus on marker-assisted selection, this talk will present some recent technological developments and discuss their impacts on an applied breeding programme.

SOME TECHNOLOGICAL DEVELOPMENTS IN BREEDING

Mechanisation at the Roseworthy wheat breeding programme

Wheat breeding in Australia undertook one of its biggest transformations following the advent of mechanisation. In 1932 the Roseworthy Agricultural College wheat breeding programme (now part of Australian Grain Technologies Pty Ltd) first started using stationary threshers to assist at harvest, but all other activities were performed manually. By the 1960’s plot harvesters were adopted, increasing the yield plot capacity of the breeding programme. This, combined with the development of the cone seeder and the adoption of computers for data analysis and processing, enabled the number of yield plots to rise dramatically (Figure 1) [1]. Increased yield plot capacity enabled a greater focus on yield selection at earlier stages in the breeding programme. Likewise, more efficient plot work led to the development of “off site” yield selection. Until then, selection was performed entirely at Roseworthy, limiting a breeder’s appreciation of, let alone ability to manage, the impacts of genotype-by-environment interaction. With improvements in mechanisation, adoption of computers, and the application of interpretive principles developed by Finlay and Wilkinson [2], multiple sites were used for early generation grain yield selection to achieve wider adaptation. These technological improvements, which led to greater efficiencies, also allowed the breeding programme to widen its scope.

Resistance to diseases such as Septoria tritici and cereal cyst nematode (amongst others) as well as improved end-use quality could now be targeted without fear of a reduction in genetic gain for grain yield [1]. This is an example of the frequently observed ripple effect of integrating new technologies. Although the technology employed may address a specific issue or bottle neck in selection, it has often led to increases in the capacity and efficiency of other aspects of the breeding programme.

The effects of computerisation on breeding logistics

Since their availability, computers have been used for the design of trials, management of field books and harvest labels, as well as data analysis. Barcodes are now used throughout the breeding programme, for data collection (grain yield, screenings, test weight and NIR quality prediction), sample processing and trial loading. All field based measurements/observations are collected electronically with custom designed software run on Pocket PCs and uploaded into a plant breeding database (Agrobase II, Agronomix Canada). The integration of barcodes and electronic data capture has saved the breeding programme substantial resources and importantly reduced the frequency of human error. This resource saving has been directly invested into a greater number of yield plots, with more traits assessed, at earlier stages in the breeding programme.
Advances in statistical analysis

Aided by rising computer power, improvements in statistical methodology have had a large impact on the effectiveness of selection, particularly for grain yield. Progression from analysis of variance, to moving mean and now spatial analysis [3] has incrementally increased trial heritability. Practically, this has led to a more efficient yield selection system. Yield nurseries now utilise a lower number of replicates (often single replicated experiments) and can therefore test a greater number of lines at more environments for the same resources. More recently, methods have been proposed to incorporate pedigree relationships within these analyses [4]. It is hoped that this will lead to another jump in experimental accuracy and allow better prediction of line breeding values for cross prediction purposes and improved selection. In the future, it may be useful to extend this principal to molecular marker data. Rather than estimate relatedness using pedigrees, markers may be used to measure relatedness, providing additional improvements in selection accuracy. Similarly, inclusion of allele specific markers in data analyses may further improve the heritability of selection and allow the breeder to “look behind” well characterised loci (such as those controlling height, phenology or disease resistance) when searching for potential parents.

The impact of out-of-season nurseries

Out-of-season nurseries have been used for a number of years in breeding programmes such as that at CIMMYT (Mexico) to speed a population’s progression toward homozygosity. At Roseworthy however, an additional generation, grown over summer, was only introduced in 2001. The cost of irrigation and bird control as well as hostile summer temperatures has previously deterred most breeders from attempting this summer generation. The biggest impact of the inclusion of these summer generations has been to decrease the time from cross to variety release by 3 to 4 years. Within the modified bulk method employed at Roseworthy the summer generation is used from F1 to F3. Selection opportunities over summer are limited to plant height and uniformity because growth habit over summer is quite different to that over winter, and the potential of establishing a green bridge for disease carry over excludes the tempting opportunity to encourage a disease epidemic. Summer generations have also allowed the breeding programme to respond quickly to changing demands by growers by fast-tracking commercial seed multiplication of advanced lines. By way of example; an advanced line was identified at the end of the 2004 season for possible commercial release. Approximately 100 single plant selections were taken and grown over summer to check for uniformity and start seed increase. In 2005 these individual selections were grown out under irrigation over winter and again checked for uniformity. The grain was harvested, uniform selections bulked and sent to Tasmania for further multiplication over summer, ready for release in the 2006 season. The end result was the production of 250t of seed for sale from approximately 200g of seed only 16 months earlier.

Doubled-haploids to speed up breeding

Doubled-haploids (DHs) were first employed by the Roseworthy breeding programme in the mid-1990’s. Although experimental at the time, and quite expensive, their adoption allowed elite crosses to be fast-tracked through the breeding programme. The reduction in time from cross to release was greatest prior to the introduction of out-of-season nurseries. Even with the benefits of out-of-season nurseries, DHs can still reduce
the time to market by one to two years. However the high cost of each DH and the lack of selection opportunities from F₁ to fixed line have limited the usefulness and application of DHs at Roseworthy. As discussed later, combining DH production with marker-assisted selection (MAS) has overcome some of these problems and allowed DHs to be better integrated within the aims of the programme. The first two DH lines from the Roseworthy programme were released in 2007. A third variety was released in 2008 and a fourth will be made available to growers in 2009.

THE INTEGRATION OF MARKER-ASSISTED SELECTION

Most plant breeders are aware of the potential benefits that selection with molecular makers can provide. Unlike phenotypic selection, genetic selection with markers is not influenced by the environment and can be performed at any growth stage. Depending on the trait being manipulated, MAS may also be cheaper than phenotypic alternatives. But as with the many technologies that preceded MAS, the biggest challenge is often integrating these benefits without diminishing the effectiveness of the remaining selection systems.

Marker-assisted selection has now become an integral part of the Roseworthy wheat breeding programme and is used primarily to increase the frequency of desirable alleles in complex F₁ populations (eg., BC₁F₁ or TC₁F₁). Markers have also been used to select elite parents for crossing, marker-assisted backcrossing, selection during DH production and identification of elite fixed lines for fast-track seed production.

Since markers were first employed in the Roseworthy wheat breeding programme, there have been dramatic changes in the laboratory methods employed. Most markers used at the beginning of MAS at Roseworthy were restriction fragment length polymorphisms, requiring large quantities of high grade DNA. Consequently, less than 100 extractions were able to be performed in a day. Now, with the shift to PCR based marker systems such as simple sequence repeats, DNA quantity and quality requirements have reduced, allowing a single person to extract up to 1000 samples a day. With subsequent developments such as fluorescent and multiplexed fragment separation and detection, and robotic fluid handling, the number of assays able to be performed has risen substantially. In 2006, over 100,000 marker assays were performed servicing the requirements of the four AGT wheat breeding programmes. This rise in capacity has forced breeders to consider how the activities of the breeding programme could be adapted to facilitate greater marker throughput within the laboratory.

A simple example of the impacts of the field programme on the efficiency of MAS is the grow out of plants ready for tissue harvest. Ideally, if a BC₁F₁ population were to be screened with markers, the seeds would be planted in a relevant field location so that elite plants within the population could be identified by both genetic and visual selection. However it is the experience of these authors that field based tissue harvest is prohibitively slow and difficult. Seeds must either be planted in perfectly regular patterns (usually by hand) or every plant tagged with a label in order to track DNA identities in the field. In addition, sampling must be done early in plant growth (too allow enough time for the marker results to be produced before maturity) which requires those sampling to work in unfavourable conditions, stooping to ground level to harvest tissue (when the plant is at 2-3 leaf stage). Consequently, when sampling from the field, tissue from around 100 plants is harvested per person per hour. This contrasts to harvesting from a glass house where a single person can harvest tissue from around 400 plants per hour. In order to save precious glass house space, plants are grown in small tubes (10cm x 7cm) arranged in supporting trays (216 plants/m²). If further crossing is to be undertaken with these plants, they are transplanted into larger pots to allow greater tillering. For the majority of MAS, where improving the frequency of desirable alleles within the population is the primary focus, plants are grown out to maturity in these pots yielding 50-100 seeds per plant. It was discussed at the early stages of MAS within the Roseworthy wheat breeding programme that plants could be arranged in the same configuration as the laboratory “plate layout” (ie 8 x 12 arrays). However in these authors’ experience this provides very little advantage at the time of tissue harvest and if anything slows seeding, wastes glass house space and slows harvest at maturity. The most important rate limiting factor is the efficient tracking of sample identity to allow rapid harvest of both tissue and seed. As marker capacity increases, and MAS can be applied at a greater number of stages in the breeding programme, similar logistical challenges are likely to be encountered. To see continued growth in MAS, both the field activities and laboratory activities must come under scrutiny for potential changes so as to increase the efficiency of the overall system.

A PRACTICAL EXAMPLE OF MAS AT ROSEWORTHY

Recently, a breeding strategy was employed at Roseworthy that integrated the use of out-of-season nurseries, DH production and marker-assisted selection in an attempt to achieve higher rates of genetic gain and reduce the time from cross to release [5, 6]. The objective of the strategy was to introgress favourable rust and quality genes from ‘Annuello’ into an agronomically elite, but rust susceptible breeder’s line ‘Stylet’. In early 2003, 72 BC₁F₁ lines were screened with molecular markers and the surviving elite plants used as DH donors. Haploids (2000) were again screened with molecular markers and 242 lines were progressed as doubled haploids. These lines were then multiplied over summer before entering preliminary yield trials in 2004. By the end of 2007 over 200t of
commercial seed of a selected individual had been produced ready for release to growers. The time from cross to commercial seed production was just five years, around seven years less than was usually the case within this breeding programme only 10 years earlier.

An analysis of this breeding strategy has shown that although screening haploids with markers prior to doubling resulted in a reduction in cost, selection within the BC,F population had the largest impact on genetic gain. Introgression of both the Lr34/Yr18 and Lr46/Yr29 loci into the susceptible recurrent parent background resulted in substantial improvements in leaf rust and stripe rust resistance levels. Likewise, when favourable glutenin alleles were selected, both dough resistance and dough extensibility were significantly improved. By increasing the frequency of the desirable alleles early in the breeding programme, expenditure was restricted to lines more likely to be retained at later selection events [5, 6]. Overall, the efficiency of this breeding strategy was high, but the overall expenditure was also much higher than that incurred by the normal breeding programme. Consequently, although this type of strategy is still employed, it is reserved for elite crosses, where chances of commercial success are high.

WHERE TO FROM HERE FOR MAS?

The current Roseworthy wheat breeding programme utilises approximately 40,000 marker data points a year (from around 10,000 DNA samples). Although this is a sharp increase on the ~2,500 assays completed in the year 2000, it is still far from what could conceivably be integrated (resources permitting) to improve the genetic gain of this breeding programme. Where might we be in another 10 years? If technology allows it, we would like to be using molecular markers at every stage of the breeding programme. All complex F1 populations would be enriched for desirable alleles, straight cross F2 populations would be screened with markers, and as fixed lines entered the nursery system they would be characterised for any economically important loci known to be segregating. The data on fixed lines could then be used for line advancement, fast-track seed production, parent identification and to improve statistical analysis of agronomic performance. This could see marker requirements surpass 500,000 assays for this programme alone. So what is required to get us to this point?

Continued genetic analysis must be the cornerstone of future MAS. Without the development of additional marker-trait associations, MAS will rapidly become conservative, only capable of selecting genes already well characterised and fixed within the breeding programme. For genetic gain to continue, and the early impacts of MAS to be extended, new genes/QTL involved in the control of economically important traits must be tagged with robust markers useful to a breeding programme. Much of this may best be performed ‘in sync’ with the targeted breeding programmes. The concept of ‘map as you go’ has been proposed as a means to ensure allele estimates remain relevant over time [7]. Likewise, as genotyping costs continue to reduce, discovery of trait-marker associations may be able to occur routinely as part of the breeding programme.

As discussed, the progression of MAS has been strongly influenced by improvements in molecular technology. For the number of marker assays servicing the Roseworthy wheat breeding programme to increase from 40,000 to 500,000 there must be a frame shift in most molecular activities. Most importantly, DNA extraction throughput must rise from 1000/person/day to a level approaching 1,000/person/hour. Marker platforms such as single nucleotide polymorphisms may need to replace the current use of SSRs to enable the required rise in marker assays. As the use of robotics and specialised marker equipment increases, we are likely to see a shift toward centralisation of marker resources due to the high capital cost of infrastructure. At present, the close relationship between the breeder and marker implementer is a critical component of successful MAS. Third party service providers may be able to provide some increases in throughput and efficiency but are unlikely to drive innovation at the interface between field and laboratory activities.

To achieve this frame shift in MAS throughput resources within the field team would need to be redirected, at least partially, from many of their normal activities (design, preparation, and management of grain yield and disease nurseries) toward increased crossing (to produce more complex F1 populations), larger and more efficient systems for plant grow-out and faster tissue harvest. Ideally, the flow of samples and data between the field and laboratory teams would be seamless.

CONCLUSION

The primary objectives of wheat breeding within Australia have not changed since its inception. Varieties must be released to growers which increase on-farm profitability by improving grain yield, end-use quality and protection against disease. However the exciting prospect for plant breeders is the opportunity to integrate new genetic knowledge, selection methodologies, and technologies within their breeding programme. Over the next few years we are likely to see the use of molecular markers continue to rise. Their potential to increase the efficiency of a breeding programme is certain. The challenge for us as breeders, is to see that potential become reality.
REFERENCES


