

## Preface

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### Background

Barley (*Hordeum vulgare*) ranks as the world's fourth major cereal crop after maize, wheat, and rice. It is also one of the world's ancient cereal crops with archaeological remains suggesting it was first domesticated in the Fertile Crescent around 10000 years ago—at about the same time as wheat. Barley is very closely related to wheat and this similarity allows the production of fertile hybrids between the two species. Despite the similarity, barley is generally regarded as an inferior staple to wheat, and 'poor man's bread'. However, barley is the hardier of the two species and this has helped ensure its continued cultivation throughout history (Zohary and Hopf 1988).

Compared with wheat, the taxonomy and evolution of barley is relatively straightforward, with the term barley only used to describe one species, *H. vulgare*. However, a number of different subspecies have been identified growing wild in and around the Fertile Crescent, and in secondary habitats from the Mediterranean to the Himalayas. Of these, the subspecies *H. vulgare* ssp. *spontaneum* is considered the progenitor of cultivated barley. This wild barley, along with a range of other *Hordeum* species, can still be found in its original habitat in the Fertile Crescent (Nilan and Ullrich 1993; Badr *et al* 2000).

### World barley production

Barley has profited from the changes that have occurred in breeding strategies and in farming practices, resulting in a steady rate of yield increases. Figure 1 shows the global changes that have occurred in the area sown to barley and the total production since 1961. Over this period, the yields of barley have risen from an average of 1.3 t/ha to >2.5 t/ha. However, it is interesting to note that since the mid 1980s the area sown to barley has been declining. This is probably related to the increasing success of new maize hybrids and soybean cultivars in the USA and to the higher value of wheat in many areas. A second factor is the reduction of barley as a traded staple. While barley yields showed rapid increase from the early 1960s until the mid 1980s there has been little real improvement in yields for the past 15 years.

This is probably related to barley being pushed out of some of the more productive cropping regions and moving further to low rainfall, stressed environments where it can outperform wheat.

### Barley in Australia

In Australia the trends in barley production have also been interesting. Over the last 10 years production has almost doubled while the area sown has only increased 1.4-fold (Table 1). Importantly, over the same period the amount of barley exported has grown by >2-fold and the value of the barley exports has increased 3.4-fold. This dramatic increase in value of the Australian barley and malt export industry is largely due to the increased quality of the Australian barley crop and the increased likelihood that the grain harvested will make malting grade. Improvements in varieties and farming practices have played a key role in this shift.

### The National Molecular Marker Program

Improvements in yield have been due to a large number of factors but improved varieties have certainly played a major role. Disease was seen as the key limitation on yield in the early 1980s and was the major focus of breeding programs; however, focus shifted to quality when Australia saw a sharp drop in its share of the premium malt market with the release of the Canadian variety 'Harrington'. This stimulated both breeding programs and barley genetics groups to seriously investigate the genetic control of malting quality. The key initial aim of the Australian National Barley Molecular Marker Program was to identify the major loci affecting malt quality with a secondary target to track disease resistance loci and tolerance to abiotic stresses. The structure of the program was to develop and map two major populations annually. Most of the major populations consisted of an Australian variety crossed with a malting quality variety from overseas or a variety, land race, or wild barley with specific desirable characteristics. Each population was to be grown for 2–3 years at multiple sites across Australia and suitable grain was to be malted from at least 3 sites. The malt was assayed for a range of quality characteristics.

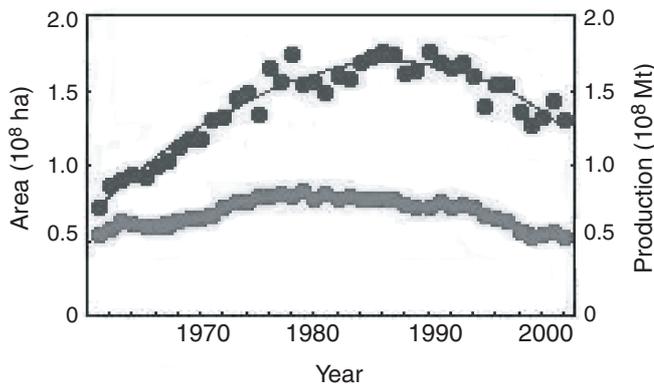


Fig. 1. World production and area sown to barley since 1961. (Data from <http://www.fao.org>)

Table 1. Barley production and export in Australia

Data from Australian Farming in Brief, 2003, Australian Bureau of Statistics

	1992	1997	2002	Fold change in 10 years
Production ('000 t)	4530	6696	8280	1.8
Area ('000 ha)	2744	3366	3707	1.4
Export—quantity ('000 t)	1964	3916	4384	2.2
Export—value (AU\$M)	297	815	1017	3.4

Table 2 shows the major populations that were developed and the source of the key overseas line. Four populations (Galleon  $\times$  Haruna Nijo, Chebec  $\times$  Harrington, Clipper  $\times$  Sahara, and Amagi Nijo  $\times$  WI 2585) were developed at the Waite Campus prior to the National Program. However, they were further studied in the National Program, especially in the initial stages. The maps and genetic analysis of the populations shown in bold in Table 2 are presented in this issue. The remaining populations are still being analysed or were found to be unsuitable for effective mapping. The selected populations were believed to cover most of the major sources of malt quality from around the world. One

further population and map is presented in this issue. This is the Mundah  $\times$  Keel population that was developed specifically to study adaptation to the Australian environment. A consensus map, incorporating the RFLP, AFLP, SSR, and candidate genes markers from 5 populations is also presented in this issue and should be an invaluable tool for further genetic studies of barley.

In addition, the program sought to use bulked segregant analysis to develop markers for key disease resistance and abiotic stress tolerance loci, where the genetic control was thought to be relatively simple. Over 50 crosses and their progeny were generated and studied in this part of the program.

### Barley breeding

The ultimate objective of the National Program was to increase the 'efficiency' of barley breeding. 'Efficiency' of a breeding program is difficult to define but will consist of many components. Importantly, an 'efficient' breeding program should, within its limited resources, regularly release cultivars that significantly increase yield and quality relative to previous cultivars. Breeding malt-quality barley has been a slow process with an average of around 14 years between the time a cross is made and a cultivar released. Much of this time is required to meet the exacting quality demands of the brewers and maltsters. Further, only a small percentage of crosses will actually lead to a new cultivar (estimated to be 1–2%). Breeding programs also need to be sufficiently flexible and pre-emptive to produce cultivars that suit changes in agronomic practices, new quality demands of consumers, and new disease threats. Changes in these areas have been evident in Australia over the past 2 decades. The increase in average yields and a move to minimum or no-till cropping has led to shifts in the spectrum and severity of diseases facing barley crops and affected the subsoil constraints to production. The high value of wheat has also acted to push barley production into the less favourable environments. We have also seen shifts in markets for malting barley and malt with higher diastatic power is now sought. Several new disease outbreaks and breakdown

Table 2. The major populations mapped and assayed for malt quality and a range of other traits

In each case an Australian variety or breeders line is crossed to an exotic variety in the categories in the left hand column. The maps and genetic analysis of populations shown in bold are presented in this issue

Group	Populations
Japanese	<b>Galleon <math>\times</math> Haruna Nijo, Amagi Nijo <math>\times</math> WI2585</b>
Canadian	<b>Chebec <math>\times</math> Harrington, WABAR2080<sup>A</sup> <math>\times</math> AC Metcalfe</b>
Winter European	<b>Sloop <math>\times</math> Halcyon</b>
Spring European	<b>Alexis <math>\times</math> Sloop, Franklin <math>\times</math> Arapiles, Tallon <math>\times</math> Patty, Tallon <math>\times</math> Scarlett, Tallon <math>\times</math> Kaputar</b>
Wild	Advanced backcross QTL (Barque $\times$ CPI 71284-48)
Landrace/primitive	<b>Clipper <math>\times</math> Sahara</b>
USA 2-row	VB9524 $\times$ ND11231

<sup>A</sup> WABAR 2080 is now registered as the cultivar Baudin.

in some widely used resistance loci has also placed pressure on breeding programs.

A key motivation for the large involvement in molecular marker development was to see if this technology could accelerate the breeding and selection process to make barley improvement programs more responsive. Importantly, could molecular markers allow for an increase in the number of traits being selected for and improve the strategies for deploying resistance genes? There were also several traits that were both very difficult and costly to screen and it was hoped that markers would make these more readily accessible to breeders. Although the original view of markers was as a replacement for conventional bioassays and other techniques for phenotyping lines, it is now clear that the technology offers many other advantages.

### Current status as reported in this volume

The path from marker development to actual use in breeding programs is not simple. This volume documents many of the key steps involved in this process. While the bulk of the papers describe the construction of the linkage maps and the localisation of loci controlling key traits, several other components of the program are also documented. These include issues related to marker application such as improving marker screening systems, and the development of robust assay procedures and tools for rapidly identifying useful markers in particular regions of the genome. Although the emphasis has been on using microsatellite or SSR markers, alternative techniques such as the use of mass spectrometry were also explored (e.g. use of MALDI-ToF MS for tracking *mlo* alleles).

During the process of mapping, considerable information was also gathered on the genetic control of many important traits. Reports are provided on the mapping, validation, and utilisation of markers for resistance to spot form and net form of net blotch (*Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*), resistance to rust (*Puccinia hordei* and *P. striiformes* f. sp. *hordei*), and resistance to scald (*Rynchosporium secalis*). The development of SSR markers suitable for tracking the aluminium tolerance locus, *Alp*, is

also described. A number of papers present the results of the quality mapping work. These include papers describing the mapping of diastatic power, grain weight and size, malt extract, pre-harvest sprouting and grain  $\alpha$ -amylase, kernel discoloration, and grain protein. Physiological traits have also been studied and a paper outlines the analysis of the timing to heading. In the process of evaluating the extensive field data for the many mapping populations, a new approach was developed for identifying quantitative trait loci (QTLs) across multiple environments. A paper describing this approach and providing examples of its use is presented.

This issue also includes overview and review articles covering the genetics of quality and disease resistance in barley.

### Marker application and where to next?

Since the key objective of the marker program was to develop tools for application to barley breeding, perhaps the best measure of success is to look at the number of markers now being used with the breeding programs to aid selection. Table 3 shows the number of loci under the categories of disease resistance, abiotic stress tolerance, quality, and other that were used within breeding programs during the 2002–03 season. An important strategy of the Australian program has been to provide resources to each of the breeding programs to allow the implementation of markers, independent of the core funding of the breeding program. This certainly accelerated the rate of application and encouraged researchers to address the issues related to application. Overall it is estimated that the actual identification of marker trait association represents less than half the work required to take markers through to actual implementation.

The large number of markers currently available and the overall success of the barley mapping programs both in Australia and overseas has raised a series of new questions that will need to be addressed in exploring the further use and development of this technology. The first question relates to new work on mapping trait loci. How many more loci are there left to discover? For example, given that malt extract and diastatic power have now been mapped in many populations in Australia and overseas (over 25 loci

**Table 3. Loci currently being tracked with molecular markers in the Australian barley breeding programs**

Resistance loci: *Yd2*, Barley Yellow Dwarf Virus; *Ha4* and *Ha2*, cereal cyst nematode; *Rrs1*, scald; *Rpt4*, spot form of net blotch; *mlo* and *Mla*, powery mildew; *Rph2* and *Rph12*, leaf rust; RWA, Russian wheat aphid. Abiotic stress loci: *Bt2*, *Bt3*, and *Bt4*, boron; *Mel1*, manganese efficiency; *Alp*, aluminium; *Fr1*, frost. Quality loci: *Bmy1*,  $\beta$ -amylase; *ME1HS*, *ME2HS*, *ME2HL*, *ME5HL*, malt extract; *Ant28*, proanthocyanidin deficient; *Wx*, waxy. Other: *n*, hullless; *denso*, dwarf; *Ppd1*, photoperiod

	Number of loci	Target loci
Disease resistance	11	<i>Yd2</i> , <i>Ha4</i> , <i>Ha2</i> , <i>Rrs1</i> , <i>Rpt4</i> , <i>mlo</i> , <i>Mla</i> , <i>Rph2</i> , <i>Rph3</i> , <i>Rph12</i> , RWA
Abiotic stress tolerance	6	<i>Bt2</i> , <i>Bt3</i> , <i>Bt4</i> , <i>Mel1</i> , <i>Alp</i> , <i>Fr1</i>
Quality	7	<i>Bmy1</i> , <i>ME1HS</i> , <i>ME2HS</i> , <i>ME2HL</i> , <i>ME5HL</i> , <i>Ant28</i> , <i>Wx</i>
Other	3	<i>n</i> , <i>denso</i> , <i>Ppd1</i>
Total	27 (18 traits)	

discovered), do we now know all the major loci affecting these traits and will further studies simply identify loci that contribute a very small proportion to the overall genetic control and are of little practical value? Similarly, do we now know the location of the major disease resistance gene clusters and should our attention now shift to mapping disease tolerance? There are also many questions related to the most appropriate technologies for marker screening. The emphasis of the work described in this volume has been on the use of SSR markers. These have been excellent for tracking individual loci or even several at once, but they are not as suitable for whole genome analysis as some of the newer marker techniques.

However, perhaps the two most important questions relate to expanding the germplasm pool being used in barley breeding and developing new breeding strategies based around the new genetic information we have for barley. With the information we now have about the location of key loci, can we accelerate the identification and introgression of novel alleles from barley landraces and wild relatives? The development of new breeding strategies will be important if we are to fully utilise the technology. Table 3 shows that we already have 27 loci of relevance to the breeding programs that can be tracked with molecular markers. For this number of loci to be tracked we need to investigate new selection techniques that place the emphasis on the identification of specific recombination events and select for key linkage blocks rather than individual traits.

### What have we learnt?

The meetings conducted to coordinate this national effort quickly became a fertile ground for debating the breeding strategies which become possible with marker technology. Early in the program we implemented marker assisted backcrossing for single major dominant genes, which quickly progressed to recessive genes, then to several genes simultaneously, then to introgression of QTLs and selection of recurrent parent genotype. Backcrossing has progressed from a genetically conservative approach, which held little attraction for many breeders, to a re-invigorated technique capable of rapid and significant genetic gains with special significance for defect elimination in elite malting barley varieties. More radical breeding strategies, such as the 'conversion' of a feed variety to a malt variety by marker

assisted introgression of superior alleles for quality from malting quality donors, are under evaluation.

Within the mainstream of pedigree and progeny breeding systems, further revolutions have occurred. Techniques for the rapid attainment of homozygosity, such as single-seed descent and doubled haploids, have become more efficient since marker assisted selection (MAS) was imposed, improving the value of the lines emerging from the laboratory into field testing. Complex crossing strategies also have become more productive since MAS was applied to 3- and 4-way cross  $F_1$ s to screen for desirable alleles from the quarter parents.

We are now able to track loci controlling quality, disease resistance, and abiotic stress tolerance in barley. We also have robust protocols for using the technology in practical breeding programs. Importantly, the first varieties bred using molecular markers at some stage in the breeding process have been released—cultivar 'Sloop SA', a backcross-derived line from Sloop with the cereal cyst nematode resistance from Chebec transferred via MAS during 3 backcrosses. The technology is now integral to all barley breeding programs in Australia. Even the most hardened sceptics acknowledge that the information gained on the genetic control and linkage relationships between key traits in barley has inestimably improved the ability of the barley breeders to make genetic progress. However, the technology continues to advance. In particular the steadily decreasing cost of marker assays and the ability to screen and track the whole genome offer exciting opportunities to move into new areas of genetic analysis and selection. In a few years we may even have the whole genome sequence of barley. How will we use this information to improve barley breeding and expand the gene pool? This should be the base for the next barley marker/genome program.

### References

- Badr A, Muller K, Schafer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, Pozzi C, Rohde W, Salamini F (2000) On the origin and domestication of barley (*Hordeum vulgare*). *Molecular Biology and Evolution* **17**, 499–510.
- Nilan RA, Ullrich SE (1993). Barley: taxonomy, origin, distribution, production, genetics, and breeding. In 'Barley chemistry and technology'. (Eds AW MacGregor, RS Bhatta) (American Association of Cereal Chemists, Inc.: St Paul, MN)
- Zohary D, Hopf M (1988) 'Domestication of plants in the old world.' (Clarendon Press: Oxford, England)