

# The Use of Wild Barley (*Hordeum vulgare* ssp. *spontaneum*) in Breeding for Quality and Adaptation.

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## Introduction

*Hordeum vulgare* ssp. *spontaneum* (wild barley) is recognised as the progenitor of cultivated barley. It occupies a diverse range of habitats from high rainfall regions to desert. Studies have shown high levels of genetic variation in wild barley and a general trend of declining genetic diversity in response to domestication and selection. However, the potential for exploiting wild barley as a source of novel genes for crop improvement essentially remains untapped. This may be attributed largely to problems with linkage drag and the time and risk associated with using wild barley in traditional breeding programs.

Wild barley has successfully been used as a source of major genes for rust and scald resistance (Feuerstein et al, 1990, Abbott et al, 1992). In this paper, we describe the utilisation of wild barley for the improvement of a simply inherited trait through the identification and introgression of a superior allele for  $\beta$ -amylase. The biochemical characterisation of the novel  $\beta$ -amylase enzyme and the development of an efficient selection strategy are presented.

The next challenge is to exploit complex quantitative traits within the wild barley gene pool. Specifically, the broad adaptation and the relationship between genetic diversity and ecogeographic parameters in wild barley, suggest it may be a source of useful genes related to adaptation and stress responses. Barley is the most widely adapted crop in the world, it is grown from the Arctic Circle and high mountainous regions to the desert fringes (Harlan 1976). In most Mediterranean-type, dryland cropping environments of the world, barley is adapted best to the marginal fringes of cropping. In contrast to this general pattern of adaptation, wheat is better adapted to marginal areas in Southern Australia. Wild and landrace barleys therefore provide one option to improve barley varieties for low rainfall areas in Southern Australia where selection within *H. vulgare* has failed to improve yield and adaptation equivalent to that achieved in more favourable environments.

Tolerance to drought stress has been a difficult trait to characterise and quantify. In addition, there are difficulties associated with identification and introgression of complex traits and these are further complicated with the use of exotic germplasm. The development of a multidisciplinary strategy to identify and introgress alleles for drought stress tolerance from wild and landrace barleys is discussed.

## Wild Barley as a Source of Major Genes for Improved $\beta$ -amylase Thermostability and Fermentability

Barley  $\beta$ -amylase (1,4- $\alpha$ -glucan maltohydrolase; EC 3.2.1.2) catalyses the liberation of  $\beta$ -maltose from the non-reducing ends of 1,4- $\alpha$ -glucans and is a key enzyme in the degradation of starch during brewing. The hydrolysis of starch produces fermentable sugars required for yeast nutrition, and the yield of fermentable sugars directly affects the level of alcohol produced. Fermentability is a critical quality parameter for brewing, particularly in systems using starch based adjuncts.

$\beta$ -amylase is a relatively thermolabile enzyme compared to  $\alpha$ -amylase and limit dextrinase (Sjoholm et al. 1995). During brewing, temperatures in excess of 63°C are employed to gelatinise starch granules, which facilitates rapid and complete starch degradation. Native barley  $\beta$ -amylase retains maximum activity up to 55°C, but its stability diminishes rapidly as temperature increases above 55°C (Thacker et al. 1992).

An assessment of genetic variation for  $\beta$ -amylase was initially conducted on a set of 150 varieties of cultivated barley. Thermostability assays in conjunction with isoelectric focusing and molecular mapping were used to identify three discrete  $\beta$ -amylase alleles (*Bmy1*- Sd2L, -Sd1, and -Sd2H) in cultivated barley. The three forms of  $\beta$ -amylase exhibit different rates of thermal inactivation in barley extracts, with low, intermediate, and high levels of thermostability respectively. This variation was shown to persist after the proteolytic processing of  $\beta$ -amylase that occurs after germination. Analysis of the relationship between  $\beta$ -amylase thermostability and fermentability in 42 commercial malt samples indicates that increased thermostability results in more efficient starch degradation (Eglinton *et al.* 1998).

One hundred and fifty four accessions of *H. spontaneum* were screened for  $\beta$ -amylase polymorphism and three novel alleles (*Bmy1*-Sd3, -Sd4, and -Sd5) were identified in addition to those detected in cultivated barley. The corresponding Sd4 and Sd5 enzymes exhibit intermediate levels of thermostability, similar to the Sd1  $\beta$ -amylase. The Sd3  $\beta$ -amylase from wild barley exhibits thermostability significantly greater than the other five allelic forms of  $\beta$ -amylase.

The thermostable Sd3  $\beta$ -amylase was purified from wild barley and compared with purified Sd2L and Sd1 enzymes. Thermal inactivation analysis of the three enzymes revealed  $T_{50}$  temperatures of 56.8°C for the Sd2L enzyme, 58.5°C for the Sd1 enzyme, and 60.8°C for the Sd3  $\beta$ -amylase from wild barley. Comparative peptide mapping with protein sequencing and electrospray mass spectrometry were used to determine the amino acid differences between the three alternative forms of  $\beta$ -amylase, which were then compared with published sequence data for the Sd2H enzyme. The amino acid substitutions are consistent with the observed differences in isoelectric point. Homology based molecular modelling of all four enzymes demonstrated several of the amino acid substitutions occur in loops exhibiting higher than average chain flexibility, and several participate in hydrogen bonding, consistent with the observed changes in enzyme stability.  $\beta$ -amylase expression is thought to be modulated by intron based gene regulation (Errkila *et al.* 1998). Examination of the gene structure of the *Bmy1*-Sd3 allele revealed a 126bp deletion in intron III, consistent with elevated gene expression.

The novel isoelectric point of the thermostable Sd3  $\beta$ -amylase has been exploited by applying isoelectric focusing as a selection tool for introgressing the *Bmy1*-Sd3 allele from wild barley. Since  $\beta$ -amylase is expressed in the developing endosperm, screening can be performed on half grains and individuals grown from the remaining half grain. This approach has been used to select heterozygous F<sub>1</sub> individuals from complex crosses, facilitating rapid introgression of the thermostable  $\beta$ -amylase into elite breeding lines.

### **Wild Barley as a Source of Genes for Adaptation**

Landraces and wild barley have been recognised by the ICARDA (International Center for Agricultural Research in the Dry Areas) barley breeding program as a rich source of genes for adaptation to environments where drought stress is common. However, the introgression of traits from such germplasm is not straightforward. These types tend to be tall, susceptible to lodging, prone to head loss and/or shattering. Previous attempts to introgress complex traits from relatively wild material have often been unsuccessful due to linkage drag. New molecular techniques are available which can improve the chances of successfully introgressing genes from wild material (Tanksley and Nelson 1996). These tools can elucidate the genetic basis of complex traits, monitor the proportion of wild and cultivated DNA, check for recombination events in crucial chromosomal regions, and allow marker assisted selection for the key traits of interest.

Tolerance to drought stress has been a difficult trait to characterise and quantify. Characterisation of the many contributing factors and their genetic basis has also proven difficult. Recent advances in mapping technology coupled to physiological and biochemical tests may enable a greater understanding of stress tolerance. Mapping of these traits allows the development of molecular markers which is an important tool for introgression.

A program has recently commenced at the Waite Institute to exploit the drought tolerant traits of wild barley and landraces to improve the yield of barley in the marginal regions of Southern Australia. The research strategy developed to identify and introgress drought stress traits can be divided into three main initiatives;

#### *(1) Germplasm Introduction and Field Evaluation:*

A key aspect of utilising exotic germplasm for crop improvement is accessing appropriate genetic material. Through the formation of a collaborative research program with ICARDA, a range of germplasm is being introduced including landraces, advanced breeding lines, improved varieties, mapping parents and populations. This material represents a large range of genetic diversity and includes lines well adapted to drought stress. In addition, a series of *H. spontaneum* / Clipper backcross lines enable a comparison of different *H. spontaneum* genotypes in a similar and adapted background.

A program of field evaluation this season consists of field trials sown at two low rainfall sites Minnipa (rainfall 330mm) and Pt Wakefield (275mm) and an observation/multiplication trial sown at Charlick (495mm). Four mapping populations have been sown at two sites in Syria at Tel Hadya (336mm) and Breda (260mm) to provide initial yield data for QTL mapping.

### (2) *Biochemical and Physiological Screening for Drought Tolerance:*

Osmotic adjustment (OA) has been identified as a priority trait in breeding for drought tolerance. Conventional techniques for monitoring plant water status are not compatible with the population sizes required for detailed genetic analyses or plant breeding. This has been a major limitation in the genetic analysis of OA, with descriptive comparisons of relatively few genotypes dominating the literature. A new method has been developed to directly quantitate the organic compounds responsible for OA. This approach is based on HPLC separation of osmolytes including sugars, polyols, proline analogues and betaines (Naidu 1998), and allows the accuracy and throughput required for germplasm screening and detailed mapping studies. This methodology is also more informative than traditional measures of OA, because it allows independent quantitation of each of the osmolytes. This will facilitate both mapping of OA and dissection of the genetic control of the synthesis of its component compounds.

A broad range of biochemical and physiological traits have been implicated in drought stress tolerance. Additional characteristics to be assessed in this project include grain yield under moisture stress, maintenance of plant height and grain size/weight across environments, early vigour, glaucousness, and maturity.

### (3) *Genetic Analysis and QTL Mapping:*

The genetic basis of drought tolerance is not well understood. A molecular mapping strategy provides a genetic analysis of the drought tolerance traits as well as an approach to testing the role of candidate genes. In addition, the difficulties associated with quantifying drought tolerance have limited the implementation of screening procedures into breeding programs. The development of molecular markers for key drought tolerance traits will provide a practical approach to selection.

A total of twelve mapping populations have been developed by ICARDA representing a range of adaptation to drought stress. The mapping parents include improved varieties, landraces and breeding lines with a strong *H. spontaneum* influence. Molecular map construction has commenced on the first population, and will consist of 200 AFLPs and 30 microsatellite anchor probes.

Although established QTL mapping techniques are powerful tools, they have several deficiencies when applied to the detection and introgression of QTL from wild germplasm. In order to access alternative genes directly from *H. spontaneum*, different strategies must be applied to circumvent the following problems:

- (1) Undesirable alleles occurring in high frequency in most wild germplasm, making the collection of meaningful data difficult (lodging, shattering, sterility, large variation in maturity).
- (2) Epistatic interactions are statistically difficult to detect, yet are likely to occur in high frequency in conventional (F<sub>2</sub>, BC<sub>1</sub> or recombinant inbred) populations.
- (3) Subtle (and often negative) pleiotropic effects may go unnoticed in conventional mapping populations due to the large genetic and phenotypic variance created by the segregation of donor alleles in high frequency.
- (4) Valuable genes may not be fully expressed or readily apparent in exotic accessions until placed into adapted backgrounds.

To overcome these problems an advanced backcross QTL analysis program is being applied (Tanksley and Nelson 1996). In this approach, QTL analysis is delayed to an advanced generation (eg BC<sub>2</sub>, BC<sub>3</sub>, etc). This overcomes agronomic problems associated with individuals within a mapping population which carry a high proportion of wild germplasm, and simultaneously allows the identification and introgression of QTL. A diverse group of eight *H. spontaneum* genotypes representing the climatic and edaphic range of wild barley have been selected as donor parents. The feed variety Barque has been selected as the recurrent parent based on desirable agronomic characteristics. BC<sub>2</sub> populations will be generated and genotyping prior to subsequent phenotypic analysis.

## Summary

The research described here illustrates the potential of wild germplasm as a source of novel genes with the successful identification and introgression of a monogenic trait from *H. spontaneum* to improve the malt quality of barley. This included detailed biochemical characterisation of the thermostable  $\beta$ -amylase, however the identification and introgression into adapted backgrounds has involved relatively simple strategies. The analysis of complex quantitative traits requires more sophisticated approaches to identify, genetically characterise, and introgress useful alleles from exotic germplasm. We have developed a multidisciplinary program that combines field evaluation of diverse germplasm in the target environment with detailed biochemical and genetic strategies to exploit the genetic potential of wild and landrace barleys.

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