

Development and Implementation of PCR Markers in Western Australia's Barley Breeding Program

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Abstract

Marker-assisted selection is being increasingly used in Western Australia's Barley Breeding program. This project is intended to develop and implement molecular markers associated with disease resistance, malting quality and other agronomic traits to accelerate the production of better barley varieties. Development of new markers and adaptation of existing markers to Western Australian breeding material is being carried out, focussing on PCR markers which allow higher screening rates than conventional methods. An automated, high throughput marker-assisted selection system is also being developed to increase rates at which doubled haploid barley lines are screened for specific traits. Additional efforts are being undertaken to pyramid multiple markers into single PCR assays. The β -amylase intron III PCR marker recently developed by the Carlsberg group was adapted to our breeding material and progress towards the development of a marker associated with *mlo*-mediated powdery mildew resistance is being achieved by analyzing DNA sequences to allow identification of genomic polymorphisms consistently associated with disease resistance.

Introduction

In order to secure and capture market share of malting barley world trade, Western Australia's barley breeding program is adopting biotechnology-related tools to accelerate the release of better malting barley varieties. We are seeking the development of varieties with better malting quality and improved agronomic characteristics. Desired malting characteristics include high plumpness, high malt extracts and high diastatic power and from an agronomic viewpoint, disease and abiotic stress resistance is highly desirable. The use of molecular markers for these desired traits will speed up the release of improved varieties.

Malting Quality

Malting quality related enzymes β -amylase, α -amylase and limit dextrinase are being examined at the genetic level to determine and assess their heredity and develop molecular markers assisting selection of progenies in crosses displaying different phenotypes. Recently, a polymorphism in the intron III of the β -amylase gene was associated with increased activity (Erkkila et al. 1998) and this marker is currently being used.

Disease Resistance

Resistance to barley yellow dwarf virus is effectively achieved by the incorporation of the *Yd2* gene. Molecular characterisation of this gene has resulted in the development of a PCR-based molecular marker for selection (Ford et al. 1998, Paltridge et al. 1998). Which is currently being implemented in the barley breeding program.

A PCR marker allowing identification of *mlo*-carrying lines is currently being developed at the WA State Agricultural Biotechnology Center. This is a recessive trait providing persistent and long term resistance to Powdery Mildew (*Erysiphe graminis* f.sp. hordei) and its presence in cultivated varieties is highly desirable. Alleles at the *Mlo* locus in Western Australian lines involved in cultivar development have been sequenced and aligned to identify DNA polymorphisms associated with powdery mildew resistance. Several single nucleotide polymorphisms (SNPs) were identified, but none are consistently associated with disease resistance. Nevertheless, these polymorphisms have been used to design allele-specific primers allowing screening of progeny for specific crosses. A 16bp deletion has been identified in cultivar Fitzgerald (susceptible) upstream of the start codon in the *Mlo* gene. This mutation is being used to screen offspring of crosses with Fitzgerald as a parent using a PCR assay revealing the deletion (134bp Vs 150bp).

High Throughput Analysis

An increasing number of PCR markers are being developed and will soon be ready to implement. In order to accelerate the implementation component of the program, a Matrix-Mill (Rumsey Loomis, New York) was recently purchased. This is used to simultaneously extract DNA from 96 plant samples in a matter of seconds. As a further method to increase throughput, multiplex PCR has been developed to determine β -amylase (Erkkila et al. 1998) and *Yd2* (Ford et al. 1998, Paltridge et al. 1998) genotypes in a single assay (Fig. 1).

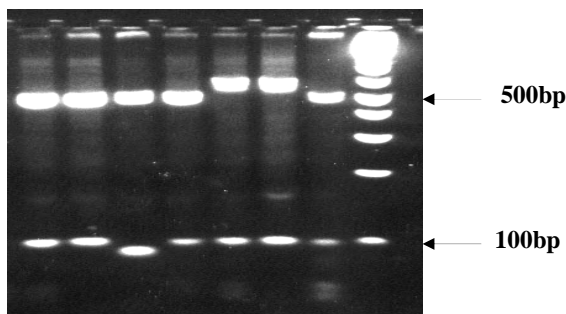


Figure 1. Examination of some of the varieties used in WA's barley breeding program - Single multiplexed PCR showing *Yd2* (small fragments ~100 bp) and β -amylase (~500 bp fragments) genotypes. Electrophoresis at 80 volts on 3% Nu-sieve agarose. Lanes (left to right) 1:Chariot; 2:Cooper; 3:Gairdner; 4:Haruna-Nijo; 5:Molloy; 6:Skiff; 7:Sloop; 8: size standard.

References

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