DArTs Without the DArT Board

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Introduction

The recently developed DArT array for oat is a promising tool for genotyping, molecular mapping, and marker/trait association studies for the development of molecular markers for marker-assisted selection (MMAS). While the DArT array can quickly and concurrently analyse thousands of loci at minimal costs per data point, the vast majority of the loci have not been linked to traits of interest. Thus the platform "over-delivers", and cannot economically meet the current MMAS requirements of most oat breeding programs.

The oat DArT probes have been sequenced and are available to the DArT Consortium members. These DNA sequences may be an interesting resource for the development of robust, PCR-based, locus-specific markers (SNPs) that may be analysed by a variety of methodologies, and which may at least in the short term, be more amenable for use in MMAS in oat.

By conversion to PCR-based allele-specific markers, we have studied selected barley DArT markers for their potential application in MMAS outside the DArT array. DArT-derived marker polymorphism rates, locus specificity and marker order conservation results, demonstrate the potential for individual DArT markers "off" the platform.

Materials and Methods

DArT probe sequences

DNA sequences for barley DArT probes bPb2058, 2881, 3921, 5252, 5610, 6421, 6457, 6659, 7755, 7998 ,8150, 8836, 9749, 9807.

Primer Design

Primers were designed to amplify as much of the sequenced fragment as possible, aided by the sequence analysis program "DNAMAN" (Lynnon Biosoft).

PCR conditions

PCR reactions consisted of 20 mM Tris-HCl, 50 mM KCl, 2 mM MgCl, 400 uM dNTP, each primer @ 200 nM, 1 unit of Taq polymerase, and 100 ng template DNA in a volume of 25 uL. Amplification cycles (35X) consisted of 94°C (45 sec.), 51°C - 64°C (45 sec.), 72°C (60 sec.).

Results and Discussion

Our study targeted a barley leaf scald resistance locus on chromosome 6HS located through very loose linkage with a poorly amplified RAPD fragment. Attempts to utilize map-based markers with tighter linkage included the analysis of SSR (48), RFLP (9), and SNP (1) candidate markers without success.

DNA sequences for 14 bPb-series DArT probes located in a 9.2cM interval flanking the locus on the DArT consensus map (Wenzl et al., 2006) and permission for their analysis, were kindly provided by Dr. Andrzej Kilian of Triticarte Pty Ltd.

- DArT probe DNA sequences ranged from 212 nt to > 850 nt (ie: no overlapping sequence when sequenced from both ends)
- Primer sets were designed to amplify as much of the DArT probe sequence as feasible
- 13 of 14 primer sets amplified robust simple banding patterns (Figure 1)
- upon PCR optimization 11 of 13 primer sets amplified single loci only
- amplified fragment lengths compared well to those expected from sequence reads

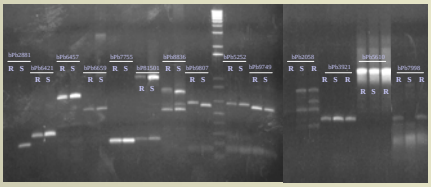

Figure 1. Composite image of amplification products for 14 DArT-derived primer sets. R = resistant genotype, S = susceptible genotype. Initial amplifications at 57 °C annealing.

- four primer sets (as initially designed) amplified polymorphic banding patterns (upon PCR optimization) (Figure 2)
- three primer sets amplified dominant polymorphisms, one primer set amplified a co-dominant polymorphism

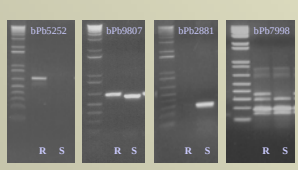

Figure 2. Polymorphic banding patterns produced by four DArT probe sequence-derived primer pairs upon PCR optimization.

- Sequencing of amplification products mediated by two additional primer pairs revealed nucleotide variation between DNA of resistant and susceptible genotypes that would allow for the design of additional SNP markers (Figure 3)
- one of these markers (bPb9749) was linked with the resistance locus, one segregated independently.

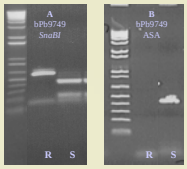

Figure 3. DArT-derived marker bPb9749 developed as a result of sequence analysis. A). Post-amplification restriction with *SnaBI* at variable nucleotide. B). Dominant allele-specific amplicon (ASA) using allele-specific primers at variable nucleotide.

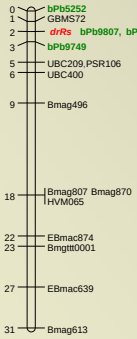

Figure 4. Linkage group of partial barley chromosome 6HS, showing location of DArT-derived markers (in green) near the scald resistance locus *drRs*.

Summary

- developed five robust PCR-based markers, two co-dominant, three dominant
- five of six markers tightly linked with scald resistance indicating locus specificity
- marker order conserved
- sequencing of monomorphic amplification products revealed additional SNPs

Results are encouraging. DArT probe sequences were long enough to increase the potential for SNP discovery but not so long as to hinder initial amplifications. DArT-derived markers showed conserved marker location and order, crucial factors when selecting mapped DArT loci for marker development. Many DArT-derived primer pairs amplified single loci making marker development simpler, an important factor when dealing with the increased genomic complexity of hexaploid oat. High polymorphism levels were promising (if not surprising) considering the DArT markers came from a region of low polymorphism for many other marker types.

While nothing can be assumed to be applied as easily in oat, DArT probes show potential for targeted (map-based) development of MMAS conducive markers.

References

Wenzl, P., Haobing, L., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, C., Xia, L., Caig, V., Ovesna, J., Cakir, M., Poulsen, D., Wang, J., Raman, R., Smith, K., Muehlbauer, G.J., Chalmers, K., Kleinhofs, A., Huttner, E., and A. Kilian. 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7, 206-227.