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PCR-based markers targeting barley putative grain yield and quality QTLs regions

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Abstract
Eight restriction fragment length polymorphism (RFLP) and two genes delimiting, or included in, chromosome fragments containing putative QTLs for grain yield and quality were sequenced and converted to PCR-markers. Eight markers were co-dominant between two-rowed barley cultivars Harrington and Baronesse after digestion with restriction enzymes. Three were dominant-recessive after designing specific primers exploiting single nucleotides polymorphisms (SNPs) between those two cultivars.

Introduction

Two chromosomal regions from Baronesse have been reported as containing putative grain yield QTLs, one on chromosome 2(2HL) (between markers ABG461C and MWG699) and the other on chromosome 3(3HL) (between markers MWG571A and MWG961) (Schmierer et al., 2004). Subsequent analysis of Harrington/Baronesse derived inbred lines suggested other additional regions as candidates for grain yield QTLs. These regions are on chromosome 7(5HL) (between markers ABC717 and ABC718) and on the telomeric region of the short arm of chromosome 2(2HS) (ABG058).

Since RFLP methodology needs large amounts of DNA and entails a complex procedure with radioactively labeled probes and Southern blotting, which requires several days to produce results, we converted RFLP targeting those putative QTL regions to PCR-markers: cleaved amplified polymorphic sites (CAPS) and SNPs. The conversion of RFLP clones to PCR-based markers rendered a much simpler technique that facilitated the screening of large numbers of genotypes at the seedling stage, since it requires a small amount of DNA.

Materials and Methods

Genomic DNA extraction was modified from Edwards et al. (1991); the modification added an extra-step of chloroform-isoamyl alcohol (24:1) extraction. CAPS were developed from RFLP clones. Primers were designed from the cloned sequences and used to amplify genomic DNA from the parental cultivars Harrington and Baronesse. If no fragment length polymorphism was observed, the fragments from both parents were sequenced to discover SNPs. These SNPs were analyzed to identify restriction enzymes that could be used to develop CAPS, or to design cultivar-specific primers. CAPS markers were also developed for gene *Dhn1* (Choi et al., 2000) and a candidate gene for seed dormancy and/or pre-harvest sprouting, *GA20-oxidase* (Li et al., 2004). Reactions for SNPs were set under stringent conditions (annealing temperatures ~ Tm) and short cycles (annealing ≤ 15 s). Extensions were 1min or 2min depending on product size.

Primer3 software (Rozen and Skaletsky, 2000) assisted the process of primer design. PCR products were visualized on 1% or 2%, depending on the fragment size, agarose gel under UV light. PCR products were purified with Exonuclease I (Exo-SAP-IT, UBS, Cleveland, OH). Sequencing reactions were performed on an Applied Biosystems 3100 Genetic Analyzer (Perkin Elmer Applied Biosystem Division, Foster City, CA) with the ABI PRISM Big Dye Terminator v3.1 cycle sequencing kit. Products were confirmed by sequencing in both directions. Analysis of sequences to find restriction sites and/or SNPs was done with the tools provided by the San Diego Super Computer Center (SDSC, http://workbench.sdsc.edu).

Results and Discussion

Details of developed PCR markers are listed in Tables 1 (CAPS) and 2 (SNPs). CAPS marker MWG699 yielded small fragments difficult to visualize after digestion with enzyme *Taq1,* even when 3% UltraPure Agarose-1000 was used to run PCR products. To avoid this problem, cultivar-specific markers were developed taking advantage of SNPs between the parental genotypes. The two SNP sites are different than the *TaqI* restriction site, for this reason they are considered two different markers for locus MWG699.

Table 1. Summary of developed CAPS markers, their location, primers and restriction enzymes

Chr.	Locus	Bin	Forward primer Reverse primer	Enzyme
3(3H)	MWG571A	009	5'-GTATCGTCAACACGGCAGCGT-3' 5'-TACCTGTCAGAAGTGCAGTACC-3'	*Bam*HI
3(3H)	MWG961	012	5'-TCAACTCCAGCCTTCACACACAAC-3' 5'-AAGACGAAGGAGACGTTGTTCATG-3'	*Bsg*I
2(2H)	MWG699	010	5'-ACCCACTGGGTTTGATACTACAAAG-3' 5'-GTGATGTTATTGGTGACTTGAACTC-3'	*Taq*I*
1(7H)	MWG851A	001	5'-CAAGAACTCCATTCCAATGTACCTG-3' 5'-TACTTCCAGATCCATGACAAGCTAC-3'	*Hae*III, *Msp*I
7(5H)	*Dhn1***	011	5'-TCACTGTTCGTACTTCGTAGCACC-3' 5'-TCCGCAGTTGCTCCTCCAAT-3'	*Taq*I, *Hpy*CH4 IV
7(5H)	ABC309	015	5'-CAGAGATACCACTGGGATTCTAAAC-3' 5'-CGAAAACCCTAGGAGAGCTAATC-3'	*Hinf*I
7(5H)	MWG2249	015	5'-AGCCATGCCGGTCTTGTCAAGAAAG-3' 5'-ATGCATCTGATCCCTGGAGAAGAAC-3'	*Alu*I
7(5H)	*GA20-oxidase*	015	5'-GTCCATCATGCGCCTCAACTACTAC-3' 5'-TAGCAAATCTTGCCATCCATCCATG-3'	*Ava*I

* Restriction enzyme (TaqI) previously reported by Tanno et al. (2002) in a different population

** Primers from Choi et al. (TAG 101:350-354)

Table 2. Cultivar-specific PCR primers developed exploiting nucleotide polymorphisms between Harrington (H) and Baronesse (B) cultivars

Chr.	Locus	Bin	Forward primer Reverse primer
2(2H)	MWG699-H*	010	5'-ATGGCTATCGCTTGACCAA-3' 5'-GTGATGTTATTGGTGACTTGAACTC-3'
2(2H)	MWG699-B*	010	5'-ATGGCTATCGTTTGACCAG-3' 5'-GTGATGTTATTGGTGACTTGAACTC-3'
2(2H)	ABG058-H	001	5'-TCTAGGCTTGCATTTGTCTACAAAG-3' 5'-ATGCTGCTTCGCTGTCTACAATAAC-3'
2(2H)	ABG058-B	001	5'-CAATAATCTCTCTTGCCATCATGCC-3' 5'-ATGCTGCTTCGCTGTCTACAATAAC-3'
7(5H)	ABC717-H*	009	5'-AACCAAGGCTACCAAGGTAATCCTG-3' 5'-CTCGTACTAACTTCCTACATGGCAA-3'
7(5H)	ABC717-B*	009	5'-AACCAAGGCTACCAAGGTAATCCTG-3' 5'-CTCGTACTAACTTCCTACATGGCAC-3'

*Note: These primers require stringent conditions when running PCR

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