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CAPS markers targeting barley *Rpr1* region

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Abstract
Eight cleaved amplified polymorphic sequences (CAPS) markers were developed around *Rpr1* genetic region. Eight markers were co-dominant between barley cultivars Morex and Steptoe after digestion with restriction enzymes.

Introduction

In barley, resistance to *Puccinia graminis* f. sp. *tritici* pathotype MCC requires the presence of at least of two host genes, *Rpg1* (Brueggeman et al., 2002) and *Rpr1* (Zhang et al., 2006). Mutational analysis and transcript-based cloning were used to isolate 3 candidate *Rpr1* genes. These 3 candidate *Rpr1* genes, HU03D17U_s_at, Contig4901_s_at and Contig7061_s_at were mapped to chromosome 4 bin 5. Screening recombinants between the three candidate genes will identify the real *Rpr1* gene. Therefore, molecular markers in this region are needed.
Cleaved amplified polymorphic sequences (CAPS) markers are PCR-based markers, requires a small amount of genomic DNA, which will facilitate the screening of large numbers of genotypes at the seedling stage. 141 probesets representing a major *Rpr1* eQTL served as a starting point to develop CAPS markers. Here we report the development and mapping of CAPS markers in the *Rpr1* region.

Materials and Methods

CAPS markers development

Plant genomic DNA extraction was modified from Edwards et al. (1991); the modification added an extra-step of chloroform-isoamyl alcohol (24:1) extraction. Barley EST unigene sequences (HarvEST assembly#21; http://harvest.ucr.edu/) were used as templates for primer design. RFLP clone MWG058 was sequenced using primers T3 and T7 with the BigDye terminator system on ABI Prizm 377 DNA sequencer (Applied Biosystems) at the Bioanalytical Center, Washington State University, Pullman. A pair of primers was designed from the MWG058 sequence. All the primer pairs listed in Table 1 were used to amplify genomic DNA from the parent cultivars Morex and Steptoe. All PCRs of 20µl contained 20-50ng of genomic DNA, 0.1mMdNTP mix, 12.5 pmol of each primer, 1µl of RedTaq DNA polymerase (Sigma), and 2 µl of 10xRedTaq reaction buffer. Amplification was performed in a PTC-100 programmable thermal controller (MJ Research, Cambridge, MA) at 95°C for 4 min, followed by 35 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min; this was followed by 7 min at 72°C. PCR products were purified using the Gel Extraction Kit (Qiagen, Valencia, CA) and sequenced. Steptoe sequence was compared to the Morex sequence in order to identify single nucleotide polymorphisms (SNPs) that could be utilized for CAPS marker development. Sequence analysis was done by VectorNTI software (Invitrogen). SNPs were identified and restriction enzymes (New England

BioLabs) were selected (Table 1). All the PCR products were digested directly using restriction enzymes correspondingly. Cleaved PCR products were then separated on 1% agarose gel.

Genetic mapping

The Steptoe x Morex "minimapper" population consisting of 35 selected doubled-haploid lines (DHL), was used to map the molecular markers to the barley Bin map (Kleinhofs and Graner 2002). CAPS marker genetic order and the distance between snp_3139 and LZ2502 was estimated based on segregation data from Steptoe x *Rpr1* F2 population.

Results and Discussion

Details of developed CAPS markers are listed in Tables 1 and Fig. 1. Molecular mapping in Steptoe x Morex population with CAPS markers showed that LZ6641, LZ13393 and LZ10152 co-segregated with LZMWG058, ABG484 and BCD453B, respectively. Markers snp_3139 (Druka, personal communication) and LZ2502 are the closest to *Rpr1* delimiting the 3 markers LZ17u, LZ4901 and LZ7061 that co-segregate with *Rpr1*. LZ2502 and sn3139 are about 1cM apart and can be used to screen recombinants in Steptoe x *rpr1* F2 population.

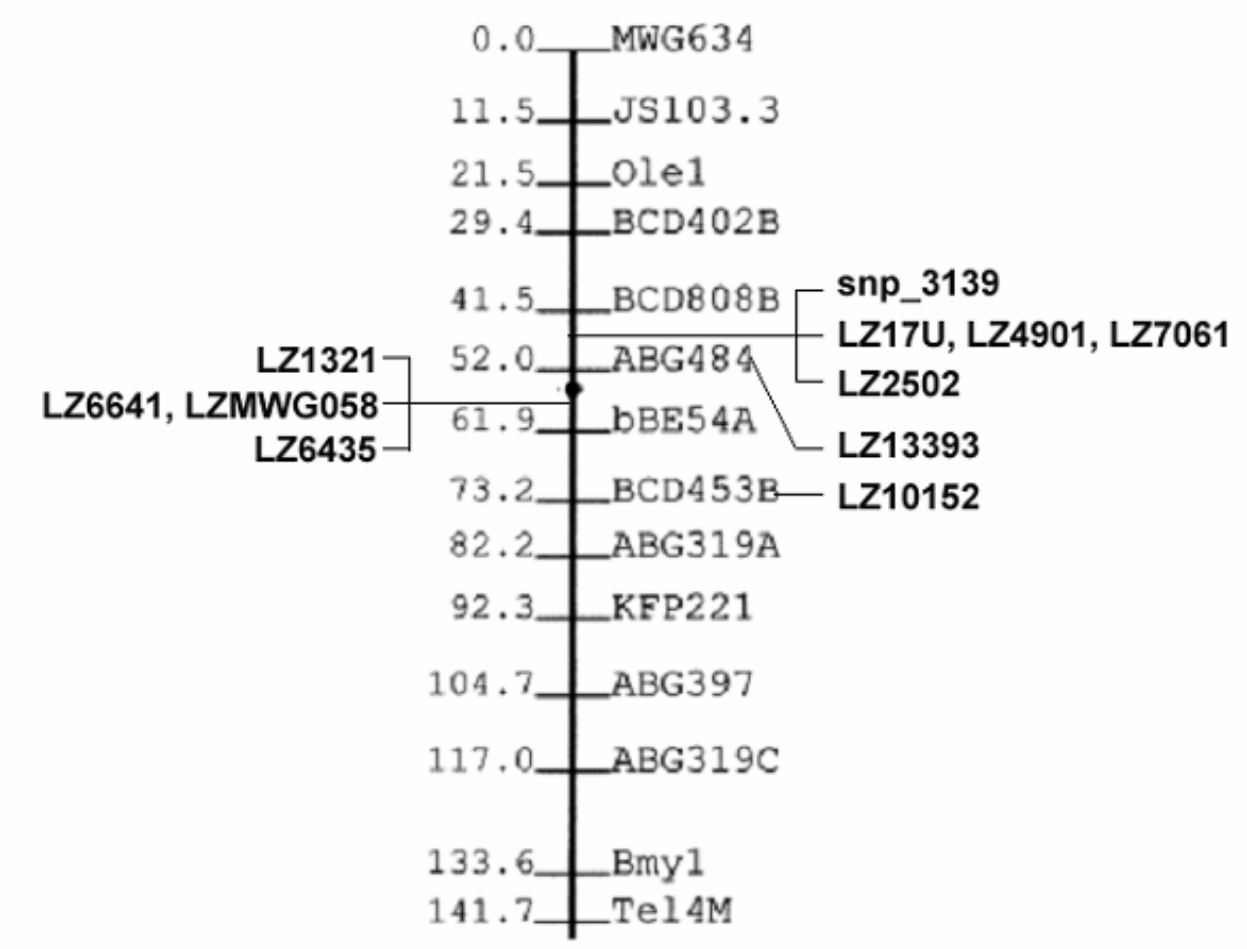

Figure 1. Chromosome locations of eight CAPS marker in barley chr. 4 (4H).

Table 1. Summary of developed CAPS markers, primers, restriction enzymes and annotation. All the CAPS markers were mapped to Chr 4 (4H), except LZ15227 was mapped to Chr 7 (5H).

Markers	Affymetrix Probesets	Forward Primer Reverse Primer	Enzyme	Annotation
LZ2502	Contig 2502_at	2502F: AGCTTCAGCTTCAGGTCGAT 2502R: GAAACTTAGAACCTGAACC	HpyCH4V	putative IAA1 protein
LZ6641	Contig 6641_at	6641F: TGATTGATCCTTTGCTGTCT 6641R: CTGGAAAGCGTTCAAATGCT	AvaII	putative expressed SLT1 protein
LZ6435	Contig 6435_at	6435F:ACACCAGGAAGATCATCGAC 6435R:ACAATGGAGAACACATGGTT	DdeI	phosphoenolpyruvate carboxy-kinase (ATP) -like protein
LZ13393	Contig 13393_at	13393F:AAGTGGACCGCGAAGCACGT 13393R:GCAGCATGTCAGGTTATACA	AvaII	hypothetical protein
LZ15227	Contig 15227_s_at	15227F:ATGGACTAATGACCCCAACA 15227R:TGCAACACACAAAGCCAGTC	AseI	microtubule associated protein
LZ1321	Contig 1321_at	1321F: CACTATCGACTTCCCGGAAT 1321R: ACTGCAATCAGGGTTCATCA	Sau3A or MboI	calmodulin
LZ10152	Contig 10152_at	10152F: AGATCTCCGGCTACGTGCTG 10152R: CGTACATCAGCTCGAAGAAA	Sau3A or MboI	putative membrane protein
LZMWG058[a]		MWG058F: ATTCATGCATCTACCCATCTCA MWG058R: TTGGATTGGCTAGAATCCTGGA	BtsI	unknown
snp_3139[b]		3139F: AACCACGCAGCAAGCCTAT 3139R: CTCGCTTCCTCCGTCATCAT	DdeI	unknown

[a]LZMWG058 was developed from RFLP clone MWG058.
[b]snp_3139 sequence provided by Druka A, Scottish Crop Research Institute (SCRI), Invergowrie, Dundee DD2 5DA, UK

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