A CAPS Marker that Distinguishes the Barley Yellow Mosaic Disease Resistance Locus *rym1* Derived from Chinese Landrace 'Mokusekko 3'

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ABSTRACT

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A Cleaved Amplified Polymorphic Sequences (CAPS) marker has been developed from a restriction fragment length polymorphism (RFLP) marker probe, MWG2134, of barley. This CAPS marker can easily distinguish varieties resistant to Barley Yellow Mosaic disease from susceptible varieties. This suggests that our CAPS marker is useful in distinguishing *rym1*-carrying varieties from non-carrying ones. The results obtained with this marker were compared with resistance tests performed on infested fields. The molecular assay corresponded well with the resistance tests in all varieties/lines and, therefore, it is helpful in distinguishing *rym1* in lines in which other *rym* genes or quantitative trait loci are present.

Key words: Barley yellow mosaic disease, CAPS marker, resistant gene, *rym1*.

INTRODUCTION

Barley Yellow Mosaic disease, caused by an RNA virus, *Barley yellow mosaic virus* (BaYMV) or *Barley mild mosaic virus* (BaMMV), is one of the most serious constraints on the production and the quality of winter barley in Asia and Europe. The most common approach to prevent infection with BaYMV and BaMMV is the introgression of the resistant genes identified in barley germplasm accessions into modern barley cultivars. In Japan, using a Chinese six-rowed barley landrace, 'Mokusekko 3', as a resistant gene donor, many BaYMV-resistant malting barley cultivars have been developed. 'Mokusekko 3' is unique in being completely resistant to all strains of BaYMV and BaMMV in Japan[7,9,13]. Konishi et al.[10] have indicated that at least two resistance genes, *rym1* and *rym5,* confer resistance to BaYMV in 'Mokusekko 3'. A restriction fragment length polymorphism (RFLP) marker linked to *rym5* has already been reported[5,6] as have the location of the *rym1* and *rym5* loci in 'Mokusekko 3'[11]. Further, Okada et al.[14] have reported that the *rym1* is completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1 and that it is acceptably resistant to BaYMV-III. However, though 'Mokusekko 3' harbours at least two BaYMV resistance genes, only *rym5* has been used for barley breeding against BaYMV; *rym1* has not been commonly introgressed into established cultivars.

Marker-assisted selection (MAS) offers the opportunity to select desirable lines based on genotype rather than phenotype, especially in the case of combining different genes in a single genotype. Several marker systems for identifying Barley Yellow Mosaic disease resistance have been reported[1,2,4–6,8,10,11,15]. However, only one study has developed a selection method for the *rym1* locus[11] and this marker system is an RFLP-based marker and, therefore, inadequate for breeders. This study describes the development of a polymerase chain reaction (PCR)-based marker system enabling the differentiation of *rym1*-carrying lines from non-carrying cultivars and its validation for molecular breeding purposes.

MATERIALS AND METHODS

PCR analysis was carried out in a sample of 10 malting barley varieties/lines. DNA was extracted from the mature leaves of the plants as described in the standard protocol of the Cethyl Trimethyl Ammonium Bromide (CTAB) method[12]. The sequence and sequence tagged site (STS) primer data of the barley RFLP probe MWG2134 were obtained from the GrainGenes database[3]. The STS primer sequences were as follows: MWG2134-1: 5′-GCA ACT CAA CGC CAT TCC AT-3′, and MWG2134-2: 5′-CCA GCG TCT TTT CAT GGG TA-3′. DNA amplifications were carried out in 50 μL reaction mixtures, containing 50 ng DNA, 200 μM dNTPs, 1 μM of each primer, 0.5 units of *Taq* DNA polymerase and 1× *Taq* polymerase buffer. Samples were heated to 95°C for 7 min and then subjected to 30 cycles of 1 min at 94°C, 2 min at 55°C, 3 min at 74°C and a final extension at 74°C for 7 min. A 5 μL sample of PCR products was individually digested with *Alu*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RV, *Hind*III, *Hinc*II, *Hinf*I, *Mbo*I, *Pst*I, *Rsa*I, *Sac*I, *Sau*3AI, *Sph*I, *Taq*I and *Xba*I, separated by 4.0% NuSieve GTG agarose gel (BioWhittaker Molecular Applications, Rockland, ME USA) electrophoresis and visualized with ethidium bromide.

Resistance tests were performed on a sample of 4 malting barley cultivars, 3 breeding malting barley lines and Chinese six-rowed barley landrace, 'Mokusekko 3'. These included the resistance gene non-carrying genotypes,

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'Amagi Nijo' and 'Haruna Nijo', the resistance genotypes 'Misato Golden' and 'Mikamo Golden' (each carrying *rym5*), our breeding lines 'Mokkei 01512' and 'Mokkei 01523' (each non-carrying *rym1*), 'Mokkei 01530' (carrying *rym1*), and 'Mokusekko 3' (carrying *rym1* and *rym5*). In the assessment of resistance to BaYMV-I, plants were grown in a field infected with only BaYMV-I and their reaction was investigated in the 2001–2002 season.

RESULTS AND DISCUSSION

As with the Barley Yellow Mosaic disease resistance genes *rym1*[11], *rym3*[15], *rym4*[1,4], *rym5*[5,11], *rym6*[8], *rym7*[5], *rym8*[2,5], *rym9*[2,5] and *rym11*[2], a RFLP/PCR marker can reliably detect the resistance and susceptible genotypes. However, reliable and available PCR markers for the detection of the *rym1* locus were not identified. This is the first report in which a reliable PCR-based marker could detect the resistant allele at the *rym1* gene locus. Oligonucleotide PCR primers, retrieved from terminal sequences of the RFLP probe MWG2134, detected a closely linked marker to the *rym1* resistance locus[11] on chromosome 4H. Sixteen restriction enzymes were screened for the detection of polymorphism between 'Haruna Nijo' and 'Mokusekko 3'. Of these, only one enzyme, *Mbo*I, showed polymorphic DNA fragments between 'Haruna Nijo' and 'Mokusekko 3'. A CAPS analysis of the DNA of the 10 malting barley cultivars/lines indicated that the two groups of genotype showed different polymorphism at the *rym1* locus. An *rym5*-carrying cultivar derived from 'Mokusekko 3' and resistance gene non-carrying genotypes carry an allele of about 102 bp; the *rym1*-carrying breeding lines of this study and 'Mokusekko 3' carry an allele of about 125 bp (Fig. 1). Subsequent segregation analysis of the CAPS marker in the 'Haruna Nijo' × 'Mokusekko 3' population showed perfect cosegregation with the RFLP marker MWG2134 (data not shown).

Within the scope of marker evaluation for molecular breeding, comparisons between field resistance and molecular test results were carried out. The infection by

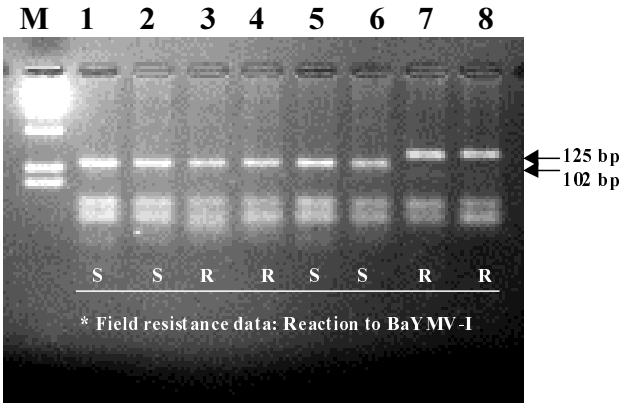

Fig. 1. Amplification and digestion of CAPS marker locus *rym1* in various barley genotypes. Amplification and *Mbo*I digests were separated in 4.0% NuSieve GTG agarose gel. Lanes: M, 100bp ladder; 1, 'Amagi Nijo'; 2, 'Haruna Nijo'; 3, 'Misato Golden'; 4, 'Mikamo Golden'; 5, 'Mokkei 01512'; 6, 'Mokkei 01523'; 7, 'Mokkei 01530'; 8, 'Mokusekko 3'.

Table I. Reaction of cultivars and lines to BaYMV-I.

Cultivars and lines	Resistant gene	Reaction to BaYMV-I (R/S)*
Amagi Nijo	—	S
Haruna Nijo	—	S
Misato Golden	*rym5*	R
Mikamo Golden	*rym5*	R
Mokkei 01512	—	S
Mokkei 01523	—	S
Mokkei 01530	*rym1*	R
Mokusekko 3	*rym1, rym5*	R

*R, resistant; S, susceptible.

BaYMV-I of barley seedlings in the field was so effective that the resistant plants were easily distinguishable from the susceptible ones. The seedlings of the susceptible controls 'Amagi Nijo' and 'Haruna Nijo' were entirely infected by BaYMV-I. However, the cultivars/lines carrying the resistance gene were not infected (Table I). In conclusion, field resistance and molecular test results showed good agreement (Fig. 1). Although 'Mokusekko 3' is completely resistant to all strains of BaYMV and BaMMV and has at least two resistant genes, *rym1* and *rym5,* only *rym5* has been utilized for breeding in Japan. In order to clarify the effect of *rym1* on BaYMV and BaMMV, and to utilize *rym1* for resistant barley breeding, we investigated only *rym1*-carrying breeding lines in their susceptibility to BaYMV and BaMMV in our previous study[14]. The results indicated that *rym1* is completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1, and is acceptably resistant to BaYMV-III[14]. We suggest that, for a new cultivar that is developed to resist these infections, a new virus resistant strain should have at least one new resistant gene, such as *rym1,* and preferably two resistant genes, to confer resistance to all strains.

The pyramiding of the BaYMV-resistant genes *rym1* and *rym5* has been practiced in our laboratory. Homozygous resistant plants are easily selected from hybrid populations between the *rym1* and *rym5* genotypes at the seedling stage in the laboratory using the CAPS marker that is linked to *rym1* (in this study) and linked to *rym5*[6]. Next, the seedlings selected by marker assistance are transplanted into the BaYMV-infested field to again prove their disease resistance. Further examination for introgression of *rym1* into the *rym5* homozygotes is made by a test cross with the *rym1* tester. These steps are laborious and time-consuming in malting barley breeding. In addition to *rym1,* we are attempting to tag other BaYMV resistance genes with molecular markers for the pyramiding of BaYMV resistance in malting barley.

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