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ORIGINAL PAPER

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rym15 from the Japanese cultivar Chikurin Ibaraki 1 is a new barley mild mosaic virus (BaMMV) resistance gene mapped on chromosome 6H

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Abstract Breeding for resistant cultivars is the only way to prevent high yield loss in barley caused by the soil-borne barley mild mosaic virus (BaMMV) complex. We have characterized the BaMMV resistance of barley cv. Chikurin Ibaraki 1. Doubled haploid lines were obtained from the F_1 between the susceptible six-rowed winter barley cultivar, Plaisant, and Chikurin Ibaraki 1. Each line was tested for reaction to BaMMV by mechanical inoculation followed by DAS-ELISA. Of 44 microsatellites that covered the genome, 22 polymorphic markers were tested on one susceptible and one resistant bulk, each comprising 30 lines. Differential markers and additional microsatellite markers in the same region were then tested on the whole population. A bootstrap analysis was used to compute confidence intervals of distances and to test the orders of the resistance gene and the closest markers. A segregation of 84 resistant/98 susceptible lines fitted a 1:1 ratio (χ^2=1.08, P=0.30), which corresponds to a single gene in this DH lines population. The resistance gene was flanked by two markers near the centromeric region of chromosome 6HS—Bmag0173, at 0.6±1.2 cM, and EBmac0874, at 5.8 ± 3.4 cM. We propose to name this new resistance gene *rym15*. This resistance gene and associated markers will increase the possibilities to breed efficiently for new cultivars resistant to the barley mosaic disease.

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Introduction

The soil-borne barley yellow mosaic disease is induced by two different bymoviruses—barley mild mosaic virus (BaMMV) and barley yellow mosaic virus (BaYMV). Both are transmitted by the soil-borne fungus *Polymyxa graminis* Led. (Toyama and Kusaba 1970), which prevents the use of chemical treatment to limit the disease. Several pathotypes have already been described for the viruses, both in Japan where the disease was first reported (Ikata and Kawai 1940) and in Europe where it was later discovered (Huth and Lesemann 1978). Seven strains of BaYMV and two of BaMMV have been reported in Japan on the basis of pathogenicity towards barley cultivars (Kashiwazaki et al. 1989; Nomura et al. 1996). Moreover, a Korean strain of BaMMV differing from the Japanese and German ones and several Chinese biological isolates of BaYMV have been recognised (Chen et al. 1996; Lee et al. 1996). In Europe, initially only one BaMMV strain and two BaYMV strains were described (Huth 1989; Huth and Adams 1990). Studies conducted in France at six different locations with a set of 34 European and Asian genotypes, however, showed that additional variants of BaMMV and BaYMV did exist (Hariri et al. 2000). More recently, a new variant of BaMMV infecting *rym5*-carrying genotypes has been characterised (Hariri et al. 2003).

Werner et al. (2003a) reviewed resistance in barley to mosaic-inducing viruses. Briefly, to date, 13 resistance

genes have been described using BaMMV mechanical inoculation or BaYMV field evaluation in Japan and in Germany. These have been localised on chromosomes 1H, 3H, 4H, 5H and 7H. For most of these resistant genes virulent virus strains have been observed. Ruge et al (2003) reported on the introgression of a dominant gene derived from *Hordeum bulbosum* (*Rym14*^{Hb}) on chromosome 6HS of *H. vulgare*. In Europe, registered cultivars carry either *rym4*, which is ineffective against BaYMV-2, or *rym5*, which has recently been shown to be not effective against a new BaMMV strain (Hariri et al. 2003). In Japan, the Chinese landrace Mokusekko 3 is still resistant to all virus strains (Iida et al. 1992; Nomura et al. 1996), but it has been shown to carry two resistant genes (Konishi et al. 1997; Okada et al. 2003). Therefore, widening the genetic basis of resistance is still necessary in order to efficiently breed for new barley cultivars.

The Japanese cultivar Chikurin Ibaraki 1 is resistant in both Germany and France to BaMMV, BaYMV and BaYMV-2 (Götz and Friedt 1993; Hariri et al. 2000) but susceptible to BaYMV in Japan (Ukai and Yamashita 1980). Early genetic studies have shown that its BaMMV resistance is not allelic to *rym4* and *rym5* (Götz and Friedt 1993), to the resistance of Taihoku A (Ordon and Friedt 1993), later described as *rym13* (Werner et al. 2003b) and to the resistance of Comte de Serre, which is supposed to carry *rym4* and *rym11* (Le Gouis et al. 2000). Ordon and Friedt (1993) also reported that Chikurin Ibaraki 1 carries only one recessive resistance gene. Werner et al. (2003a) studied the resistance of this cultivar using doubled haploid (DH) lines derived from a complex breeders cross and microsatellite markers. They were able to identify a BaYMV/BaYMV-2 resistance locus on chromosome 5H that is either allelic to *rym3* or closely linked to the latter. It was not possible, however, to characterize the BaMMV resistance gene in this population as it was probably lost during population development.

The objective of the investigation reported here was to characterise the BaMMV resistance gene of Chikurin Ibaraki 1 using a population of DH lines and simple sequence repeat (SSR) markers.

Materials and methods

The Japanese six-rowed cultivar Chikurin Ibaraki 1 was crossed to the French susceptible six-rowed winter barley cultivar Plaisant. The DH population comprising 217 lines derived from the F_1 by anther culture was provided by Dr. P. Forgeois, Institut de Genech, France. The reaction of each DH line to BaMMV was assessed in two separate experiments by mechanical inoculation of ten plants followed by DAS-ELISA (for details see Friedt 1983; Ordon and Friedt 1993). A line was considered to be resistant or susceptible when results were consistent over all of the tests, otherwise it was considered uncertain and scored as missing data.

DNA was isolated from seedlings of the two parents and of the DH lines using the NucleoSpin Multi-96 plant kit (Macherey-Nagel, Düren, Germany). Based on BaMMV phenotypic data, one susceptible and one resistant bulk, each comprising 30 DH lines, were constructed for bulk segregant analysis (Michelmore et al. 1991). Forty-four markers from Liu et al. (1996) chosen to represent each chromosome arm were tested for polymorphism between the two parents. Twenty-two of these were polymorphic and were tested on the two bulks. When identified, differences between the two bulks were checked on the 60 DH individuals of the bulks and, subsequently, on the whole population. Additional markers from Ramsay et al. (2000) were added to the map in the region of the gene.

PCR reactions were performed in a total volume of 25 µl in 9700 Applied Biosystems (Foster City, Calif.) thermocyclers. The reaction mixture contained 20 ng genomic DNA, 1× GeneAmp PCR Buffer II, 1.5 m*M* $MgCl_2$, 1 U AmpliTaq Gold (Applied Biosystems), 250 μM of each dNTP, 0.05 μM R110-5-dCTP (NEN Life Science Products), 0.2 μM of each primer. The PCR conditions for each primer set were the same used by Liu et al. (1996) and Ramsay et al. (2000) except that we applied an initial phase of 94°C for 11 min for enzyme activation.

Prior to electrophoresis, 1–2 μl of the PCR-amplified sample was mixed with 19.5 μl Hi-Di formamide and 0.5 μl Genescan-400HD ROX (6-carboxy-x-rhodamine) internal size standard, denatured at 95°C for 3 min and kept on ice until loading. SSR fragment analysis was carried out on an ABI Prism 310 Genetic Analyser equipped with the GeneScan 3.1 analysis software. Alternatively, IRD700- and IRD800-labelled primers were used, and the PCR products were separated on an 8% Long Ranger Gel (FMC Biozym, Hessisch Oldendorf, Germany) and detected on a LI-COR DNA Sequencer Genreadir 4200 (MWG Biotech AG, Ebersberg, Germany; Werner et al. 2003a).

Linkage analysis was performed with the MAPMAKER VER. 3.0 software (Lander et al. 1987) using the Kosambi function (Kosambi 1944) to estimate map distances and the "error detection on" option to detect typing errors. Order and confidence intervals for distances between the three closest markers and the resistance gene were calculated using a bootstrap strategy with 1,000 random bootstrap samples of the same population size drawn with replacement (Liu 1998).

Results

The resistance test involving mechanical inoculation of BaMMV suggested the presence of one resistance gene. Segregation of 84 resistant:98 susceptible lines fitted a 1:1 ratio (χ^2=1.08, P=0.30), which corresponds to the presence of a single gene in this DH population.

In order to localise this BaMMV gene, two DNA bulks were constituted and analysed using a set of microsatellites. Primary screening revealed a difference between the two bulks for markers HVM14 and HVM74 localised on barley chromosome 6H. Individual tests, first on the constituent of the bulk then on the whole population, confirmed a linkage between the resistance gene and these markers on chromosome 6H (data not shown). A significant segregation distortion was observed for these two markers with an excess of the Plaisant allele (Fp=0.60, χ^2=9.22, P=0.002). Based on the map of Ramsay et al. (2000), additional markers were selected in the centromeric region of chromosome 6H (EBmac0806, Bmg0001, Bmac0127, Bmag0009, Bmac0018, Bmag0867, Bmag0870, EBmac0874, EBmac0639, Bmag0496, Bmag0173), and these were analysed in the DH population. The same significant segregation distortion was observed at all of the markers' loci, with an excess of the Plaisant allele (Fp=0.6 on average). The linkage analysis on the whole population and all of the markers yielded the map presented in Fig. 1, which is compared to the Lina × *H. spontaneum* Canada

Table 1 Sizes of the alleles of the three markers closest to the resistance gene

Marker name	Distance to *rym15* (cM)	Chikurin allele (bp)	Plaisant allele (bp)
Ebmac0874	5.8±3.4	172	195
Bmag0173	0.6±1.2	130	124
Ebmac0639	1.6±1.8	139	159

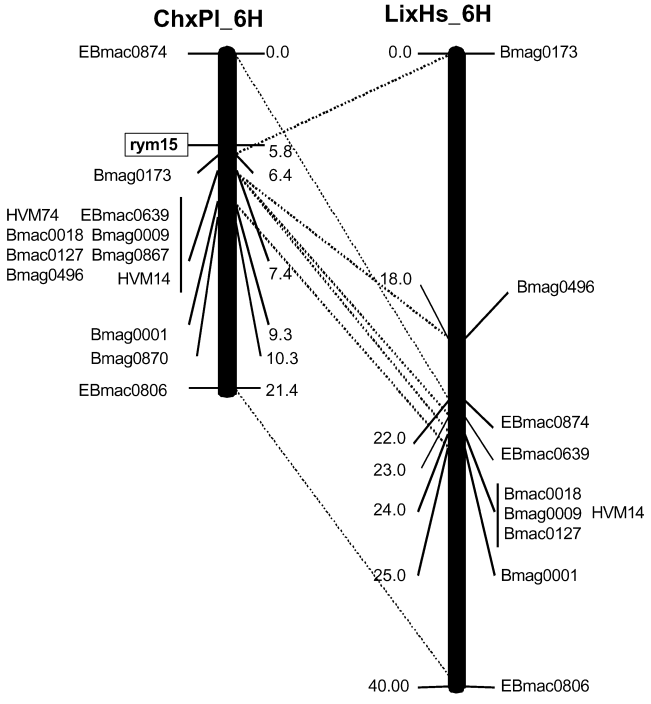

Fig. 1 Genetic map of barley chromosome 6H including BaMMV resistance gene *rym15* of cv. Chikurin Ibaraki 1 based on the analysis of 217 DH lines derived from a Chikurin Ibaraki 1 × Plaisant (*Ch*×*Pl*) cross and its comparison with the Ramsay et al. (2000) map from Lina × *Hordeum spontaneum* Canada Park (*LxHs*)

Park map constructed by Ramsay et al. (2000). The Chikurin Ibaraki 1 × Plaisant partial map spanned 21.4 cM around the centromeric region of chromosome 6H. The BaMMV gene is flanked by the two markers Bmag0173, at 0.6±1.2 cM, and EBmac0874, at 5.8±3.4 cM (Table 1). The most probable orders of the resistance gene and the three closest markers were tested with a bootstrap procedure (Liu 1998). This analysis showed that the BaMMV gene is localised between the two markers Bmag0173 and Ebmac0874 in 76.5% of the bootstrap samples. It is localised between Ebmac0639 and Ebmac0874 in 85.2% of the samples.

Discussion

The Japanese barley cultivar Chikurin Ibaraki 1 has been shown to be resistant in Germany to BaMMV, BaYMV and BaYMV-2 (Götz and Friedt 1993). We can now conclude that this pattern of resistance is due to one gene on chromosome 5HS that is efficient against BaYMV/BaYMV-2 (Werner et al. 2003a) and one gene on chromosome 6HS that is efficient against BaMMV. Ruge et al (2003) reported the introgression in *Hordeum vulgare* of a dominant gene from *H. bulbosum* named *Rym14*^{Hb}. This large introgression of 21 Mb is located at the telomeric end of chromosome 6HS. No other soil-borne mosaic resistance gene has yet been assigned to the centromeric region of chromosome 6H (Werner et al. 2003a), and we propose to name this gene *rym15*.

We found segregation distortion at all marker loci in the centromeric region of chromosome 6H. Such a situation is common in DH populations produced by anther culture (Graner et al. 1991; Zivy et al. 1992). Devaux et al. (1995) and Manninen (2000) reported segregation distortions at loci located on chromosome 6H in anther culture-derived DH populations. It is possible that the distorted areas may contain genes involved in anther culture response (Devaux and Zivy 1994), but this has to be further investigated. The marker order of the partial map of Chikurin Ibaraki 1 × Plaisant is globally the same as that reported by Ramsay et al. (2000) with the Lina × *H. spontaneum* Canada Park population composed of 86 DH lines (Fig. 1). HVM14, Bmac0018, Bmag0009 and Bmac0127 co-segregated in both populations. The order of the two flanking markers, EBmac0874 and Bmag0173, is however inversed between the two maps, with a distance of 6.4 cM in Chikurin Ibaraki 1 × Plaisant expanded to 22 cM in Lina × *H. spontaneum* Canada Park. Inversions and a reduced map length relative to the situation found on the map of Ramsay et al. (2000) were also found on chromosome 5H in another DH line population involving Chikurin Ibaraki 1 (Werner et al. 2003a). Additional mapping data are necessary to resolve this discrepancy between the two crosses. However, markers located distantly from the centromeric region and therefore suitable for obtaining information whether or not reduced recombination covers the whole chromosome in the Chikurin Ibaraki 1 × Plaisant population—for example, Bmac0316 and Bmac0251—turned out to be monomorphic. The most important thing from a practical point of view is whether the majority of plants selected on the basis of these markers will carry the resistance-encoding allele. This can be achieved by choosing the two flanking markers Bmag0173 and EBmac0874 instead of only one of the two markers. The confidence can be increased by choosing the EBmac0639 marker instead of the closer Bmag0173 as shown by the bootstrap procedure. The two markers Bmag0173 and EBmac0874 were shown to have high diversity indices, 0.72 and 0.62, respectively (Ramsay et al. 2000), which make them powerful tools for marker-assisted introgression. EBmac0639 is less polymorphic in European barley

material as its diversity index is only 0.13 (Ramsay et al. 2000).

Using two flanking markers to select for the resistance gene will lead to the selection of a large part of the Chikurin Ibaraki 1 genome. This cultivar has been shown to have a very poor yield under European conditions (Ordon et al. 1997), and so many undesirable genes could be selected by linkage drag. Resistant lines may be lower yielding when the disease is absent, as already shown for *rym4* (Le Gouis et al. 1999). The markers available are sufficient for the first steps of marker-assisted selection, but closer markers would be desirable. However, several of the SSR markers on chromosome 6H tested in addition to those listed in Fig. 1 could not be used in the DH-population analysed because they are monomophic, i.e. Bmac0316, Ebmate0028, Bmag0219 and Bmac0251. Moreover, this population showed a reduced recombination rate in this area compared to the Lina × *H. spontaneum* Canada Park (Fig. 1). As strong clustering around the 6H centromere has been described (Ramsay et al. 2000; Li et al. 2003), it is not certain that additional microsatellites in this region will really lead to a better mapping and the development of closer linked markers.

Dissecting the resistance to soil-borne mosaic viruses has shown that many barley landraces carry more than one resistance gene. This is the case for the Japanese landrace Mokusekko 3, which carries *rym1* and *rym5* (Konishi et al. 1997; Okada et al. 2003) and for the old French barley landraces Champagne, Comte de Serre and Superchampenois, which probably carry *rym4* and *rym11* (Le Gouis et al. 2000). Mokusekko 3 is resistant to all pathotypes in Japan, and Superchampenois is resistant to BaMMV, BaYMV and BaYMV-2. Although these landraces are not cultivated on large areas and so are not likely to force the selection of new virus pathotypes, this tends to favour the idea that long-standing resistance against highly variable viruses can only be achieved with the combination of several resistance genes in the same cultivar. Most resistance genes have now been mapped with molecular markers. In addition, easy-to-use microsatellite and STS markers have been reported for *rym4/rym5* (Graner et al. 1999), *rym9* (Werner et al. 2000b), *rym11* (Bauer et al. 1997), *rym13* (Werner et al. 2003b), $Rym14^{Hb}$ (Ruge et al. 2003) and the BaYMV resistance gene of Chikurin Ibaraki 1 (Werner et al. 2003a). This will enable the pyramiding of barley mosaic resistance genes as proposed by Werner et al. (2000a).

References

Bauer E, Weyen J, Schiemman A, Graner A, Ordon F (1997) Molecular mapping of novel resistance genes against barley mild mosaic virus (BaMMV). Theor Appl Genet 95:1263–1269

Chen J, Adams MJ, Zhu FT, Wang ZQ, Chen J, Huang SZ, Zhang ZC (1996) Responses of foreign barley cultivars to barley yellow mosaic viruses at different sites in China. Plant Pathol 45:1117–1125

Devaux P, Zivy M (1994) Protein markers for anther culturability in barley. Theor Appl Genet 88:701–706

Devaux P, Kilian A, Kleinhofs A (1995) Comparative mapping of the barley genome with male and female recombination-derived, doubled haploid populations. Mol Gen Genet 249:600–608

Friedt W (1983) Mechanical transmission of soil-borne barley yellow mosaic virus. Phytopathol Z 106:16–22

Götz R, Friedt W (1993) Resistance to the barley yellow mosaic virus complex—differential genotypic reactions and genetics of BaMMV-resistance of barley (*Hordeum vulgare* L). Plant Breed 111:125–131

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fishbeck G, Wenzel G, Hermann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Graner A, Streng S, Kellermann A, Schiemann A, Bauer E, Waugh R, Pellio B, Ordon F (1999) Molecular mapping and genetic fine-structure of the rym5 locus encoding resistance to different strains of the barley yellow mosaic complex. Theor Appl Genet 98:285–290

Hariri D, Meyer M, Le Gouis J, Bahrman N, Fouchard M, Forget C, Andre A (2000) Characterisation of BaYMV and BaMMV pathotypes in France. Eur J Plant Pathol 106:365–372

Hariri D, Meyer M, Prud'homme H (2003) Characterization of a new barley mild mosaic virus pathotype in France. Eur J Plant Pathol 109:921–928

Huth W (1989) Ein weiterer Stamm des Barley yellow mosaic virus (BaYMV) gefunden. Nachrichtenbl Dtsch Pflanzenschutzdienstes 41:6–7

Huth W, Adams MJ (1990) Barley yellow mosaic virus (BaYMV) and BAYMV-M: two different viruses. Intervirology 31:38–42

Huth W, Leseman D (1978) Eine für die Bundesrepublik neue Virose an Wintergerste. Nachrichtenbl Dtsch Pflanzenschutzdienstes 30:184–185

Iida Y, Watanabe K, Toshima I, Ogawa K (1992) Reaction of barley (*Hordeum vulgare* L.) cultivars and lines to barley yellow mosaic virus strains. Jpn J Breed 42:863–877

Ikata S, Kawai I (1940) Studies on wheat yellow mosaic disease. Noji Kairo Shiryo 154:1–123

Kashiwazaki S, Hayano Y, Minobe Y, Omura T, Hibino H, Tsuchizaki T (1989) Nucleotide sequence of the capsid protein gene of barley yellow mosaic virus. J Gen Virol 70:3015–3023

Konishi T, Ban Y, Iida Y, Yoshimi R (1997) Genetic analysis of disease resistance to all strains of BAYMV in a Chinese barley landrace, Mokusekko 3. Theor Appl Genet 94:871–877

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–178

Le Gouis J, Ordon F, de Froidmont D, Béghin D, Jestin L, Joseph J-L, Froidmont F (1999) Agronomic comparison of two sets of SSD lines differing for the ym4 resistance gene against barley mosaic viruses. Agronomie 19:125–131

Le Gouis J, Hariri D, Bahrman N, Jestin L (2000) Resistance of old French barley cultivars to barley mosaic viruses. Z Planzenkr Pflanzenschutz 107:33–438

Lee K-J, Kashiwazaki S, Hibi T, So I-Y (1996) Properties and capsid protein gene sequence of a Korean isolate of barley mild mosaic virus. Ann Phytopathol Soc Jpn 62:397–401

Li JZ, Sjakste TG, Röder MS, Ganal MW (2003) Development and genetic mapping of 127 new microsatellite markers in barley. Theor Appl Genet 107:1021–1027

Liu BH (1998) Statistical genomics: linkage, mapping and QTL analysis. CRC press, Boca Raton, New York

Liu ZW, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor Appl Genet 93:869–876

Manninen OM (2000) Associations between anther-culture response and molecular markers on chromosomes 2H, 3H and 4H of barley (*Hordeum vulgare* L.) Theor Appl Genet 100:57–62

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant

analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Nomura K, Kashiwazaki S, Hibino H, Inoue T, Nakata E, Tsuzaki E, Okuyama S (1996) Biological and serological properties of strains of barley mild mosaic virus. J Phytopathol 144:103–107

Okada Y, Kashiwazaki S, Kanatani R, Arai S, Ito K (2003) Effects of barley yellow mosaic disease resistant gene *rym1* of the infection strains of barley yellow mosaic virus and barley mild mosaic virus. Theor Appl Genet 106:181–189

Ordon F, Friedt W (1993) Mode of inheritance and genetic diversity of BaMMV resistance of exotic barley germplasms carrying genes different from 'ym4'. Theor Appl Genet 86:229–233

Ordon F, Schiemann A, Friedt W (1997) Assessment of the genetic relatedness of barley accessions (*Hordeum vulgare* L.) resistant to soil-borne mosaic inducing viruses (BaMMV, BaYMV, BaYMV-2) using RAPDs. Theor Appl Genet 94:325–330

Ramsay L, Macaulay M, degli Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Ruge B, Linz A, Pickering G, Greif P, Wehling P (2003) Mapping of *Rym14*^{Hb}, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley. Theor Appl Genet 107:965–971

Toyama A, Kusaba T (1970) Transmission of soil-borne barley yellow mosaic virus .2.Polymyxa graminis Led as vector. Ann Phytopathol Soc Jpn 36:223–229

Ukai Y, Yamashita A (1980) Induced mutation for resistance to barley yellow mosaic virus. Jpn J Breed 30:125–130

Werner K, Friedt W, Ordon F (2000a) Strategies for pyramiding resistance genes against the barley yellow mosaic complex based on molecular markers and DH-lines. In: Logue S (ed) 8th Int Barley Genet Symp, vol 2. Department of Plant Science, Adelaide University Press, Adelaide, Australia, pp 200–202

Werner K, Pellio B, Ordon F, Friedt W (2000b) Development of an STS marker and SSRs markers suitable for marker-assisted selection for the BaMMV resistance gene rym9 in barley. Plant Breed 119:517–519

Werner K, Fried W, Laubach E, Waugh R, Ordon F (2003a) Dissection of resistance to soil-borne yellow-mosaic-inducing viruses of barley (BaMMV, BaYMV, BaYMV-2) in a complex breeders cross by SSRs and simultaneous mapping of BaYMV/BaYMV-2 resistance of Chikurin Ibaraki 1. Theor Appl Genet 106:1425–1432

Werner K, Rönicke S, Le Gouis J, Friedt W, Ordon F (2003b) Mapping of a new BaMMV-resistance gene derived from the variety Taihoku A. J Plant Dis Protect 110: 304–311

Zivy M, Devaux P, Blaisonneau J, Jean R, Thiellement H (1992) Segregation distortion and linkage studies in microspore-derived double haploid lines of *Hordeum vulgare* L. Theor Appl Genet 83:919–924