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ORIGINAL PAPER

Genetic analyses of BaMMV/BaYMV resistance in barley accession HOR4224 result in the identification of an allele of the translation initiation factor 4e (*Hv-eIF4E*) exclusively effective against *Barley mild mosaic virus* (BaMMV)

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Abstract
Key message **Based on a strategy combining extensive segregation analyses and tests for allelism with allele-specific re-sequencing an Hv-eIF4E allele exclusively effective against BaMMV was identified and closely linked markers for BaYMV resistance were developed.**
Abstract Soil-borne barley yellow mosaic disease is one of the most important diseases of winter barley. In extensive screenings for resistance, accession 'HOR4224' being resistant to three strains of *Barley mild mosaic virus* (BaMMV-ASL1, BaMMV-Sil, and BaMMV-Teik) and two strains of *Barley yellow mosaic virus* (BaYMV-1 and BaYMV-2) was identified. Analyses using Bmac29, being to some extent diagnostic for the *rym4/5* locus, gave hint to the presence of the susceptibility-encoding allele at this locus. Therefore, 107 DH lines derived from the cross 'HOR4224' × 'HOR10714' (susceptible) were screened for resistance. Genetic analyses revealed an independent inheritance of resistance to BaMMV and BaYMV ($\chi^2_{1:1:1:1} = 5.58$) both encoded by a single gene (BaMMV $\chi^2_{1:1} = 0.477$; BaYMV $\chi^2_{1:1} = 0.770$). Although Bmac29 indicated the susceptibility-encoding allele, BaMMV resistance of 'HOR4224' co-localized with *rym4/rym5*. The BaYMV resistance was mapped to chromosome 5H in the region of *rym3*. Sequencing of full length cDNA of the *Hv-eIF4E* gene displayed an already sequenced allele described to be efficient against BaMMV and BaYMV. However, the F_1 progenies of crosses involving 'HOR4224' and *rym4/rym5* donors were all resistant to BaMMV but susceptible to BaYMV. Therefore, this is the first report of an allele at the *rym4/rym5* locus exclusively efficient against BaMMV. Changes in the specificity are due to one non-synonymous amino acid substitution (I118K). Results obtained elucidate that combining extensive segregation analyses and tests for allelism involving different strains of

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BaMMV/BaYMV in combination with allele-specific re-sequencing is an efficient strategy for gene and allele detection in complex pathosystems.

Introduction

The viruses of the barley yellow mosaic complex (*Barley mild mosaic virus* and *Barley yellow mosaic virus*), belonging to the *Bymovirus* genus in the family *Potyviridae*, are the most important soil-borne viral pathogens of winter barley due to a constant spread and high-yield losses frequently observed in susceptible cultivars. Bymoviruses are transmitted via the soil-borne plasmodiophorid *Polymyxa graminis* (Toyama and Kusuba 1970; Adams et al. 1988), and chemical measures to control this vector are neither economically nor environmentally acceptable. Therefore, breeding and growing of yellow mosaic disease-resistant barley cultivars are considered to be the only practical way of preventing high-yield losses.

Recessive inheritance as the common mode of resistance to potyviruses (Lopez-Moya and Garcia 2008) is based on a lack of fully functional host factors vital for a particular step in the virus life cycle (Fraser 1990; Revers et al. 1999). In this regard, mutations in genes of the eukaryotic translation initiation complex were identified as underlying components of potyvirus resistance in many plant species (Robaglia and Caranta 2006; Maule et al. 2007; Yeam et al. 2007). In barley, so far, 18 resistance genes corresponding to at least 9 different genetic loci in most cases also confer recessive resistance to either BaYMV or BaMMV, or even to several strains of both bymovirus species (Kai et al. 2012; Ordon and Perovic 2013).

In Europe, barley breeding for resistance to barley yellow mosaic disease has relied mainly on the selection of the recessive resistance gene *rym4* and more recently also on *rym5*, an allele of *rym4* providing more broad spectrum resistance against bymoviruses. The *Rym4/Rym5* locus has been shown to correspond to the eukaryotic translation initiation factor 4E (*Hv-eIF4E)* gene (Kanyuka et al. 2005; Stein et al. 2005) where *rym4* and *rym5* alleles contain specific missense mutations in the coding region of *Hv-eIF4E*. The rapid and wide spread of barley yellow mosaic disease and the occurrence of new virulent bymovirus strains potentially cause an increased threat to barley production in coming years (Kühne 2009). Since the genetic base of resistance to this disease in European barley cultivars is largely based on *rym4/rym5*, and due to the occurrence of new resistance breaking BaMMV and BaYMV strains (Hariri et al. 2003; Habekuss et al. 2008) it is important to search for new effective sources of resistance.

The identification of the chromosomal location of resistance genes and the development of selectable molecular markers are the first steps towards the efficient manipulation of disease resistance in breeding programs (Miedaner and Korzun 2012). Since many agronomically important genes have been isolated, the exploration of their allelic diversity on the sequence at the molecular level and the utilization of novel superior alleles through the development of improved cultivars using targeted molecular breeding ('precision breeding', McCouch 2004) are of prime interest. The analysis of natural allelic variation and identification of unknown valuable alleles at loci of known function are called 'allele mining' (Kaur et al. 2008). Allele mining on the *Hv-eIF4E* gene yielded the identification of novel alleles (Hofinger et al. 2009, 2011; Ling et al. 2009).

In case that a resistance gene is cloned, it is straightforward to query this gene sequence in collections of natural genetic diversity. However, with respect to BaMMV/BaYMV it has to be taken into account that nine different resistance loci are known and that, therefore, an allele mining at the *Hv-eIF4E* locus may be of limited value as it is not possible to assign newly detected alleles unequivocally to resistance. Therefore, genetic mapping and tests for allelism followed by targeted re-sequencing are a prerequisite to detect new alleles in such pathosystems (Boisnard et al. 2007; Perovic et al. 2007).

A field screening program has previously been carried out using the barley germplasm collection of the Institute of Plant Genetics and Crop Plant Research Gatersleben (IPK) aimed to identify winter barley accessions with resistance to bymoviruses (Proeseler et al. 1989, 1999). The identified resistant accessions have been additionally sifted through using the diagnostic SSR marker Bmac29 to identify those that do not carry *rym4* or *rym5* (Graner et al. 1999; Habekuß et al. 2000). Selected resistant genotypes were then crossed to known susceptible cultivars and F_1 derived doubled haploid (DH) populations were produced. Based on these, the main aims of the current study were to investigate (1) the genetic basis of resistance to barley yellow mosaic disease in 'HOR4224', and (2) to develop molecular markers for corresponding disease resistance genes facilitating efficient marker-assisted breeding.

Materials and methods

Plant material

A population of 107 DH lines derived from a cross between the IPK Gene Bank (http://gbis.ipk-gatersleben.de/gbis_i/) winter barley accessions 'HOR4224' ['Aizn Coiled Necn', *Hordeum vulgare* L. convar. *vulgare* var. *parallelum* Körn. (resistant)] and 'HOR10714' ['Monroe', *Hordeum vulgare* L. convar. *intermedium* (Körn.) Mansf. var. *japonicum* (Vavilov & Orlov) Mansf. (susceptible)] by the

use of the '*Hordeum bulbosum*' technique (Pickering and Devaux 1992) was investigated for resistance to BaMMV and BaYMV. Test crosses for determining allelism were made between 'HOR4224' and cultivars 'Carola' (*rym4*), 'Express' (*rym4*), 'Tokyo' (*rym5)*, 'Ea52' (*rym3*) and 'Chikurin Ibaraki 1' [*rym15* (Le Gouis et al. (2004) and another unnamed BaYMV resistance gene, which is proposed to be allelic to *rym3* (Werner et al. 2003)]. The resistance pattern of accessions carrying different *Hv-eIF4E* alleles, i.e., a standard set of four cultivars: 'Franka' (*rym4*), 'Tokyo' (*rym5*), 'Miho Golden' (*rym6*), and 'Hiberna' (*rym10*), was compared with the resistance reaction of parental lines from this study 'HOR4224' (resistant) and 'HOR10714' (susceptible). The four cultivars mentioned above carrying different alleles of *Hv-eIF4E* were tested and used as the standard controls along all performed experiments according to Habekuss et al. (2008).

Resistance tests

Resistance tests on the 107 DH lines, parental lines 'HOR4224' and 'HOR10714', and four cultivars carrying *Hv-eIF4E*-based resistance (see above) were carried out in Lower Saxony, Germany on naturally infested fields at Eikeloh (BaMMV and BaYMV-2) and on two artificially contaminated fields in Saxony-Anhalt at Aschersleben (BaMMV/BaYMV-1 and BaMMV/BaYMV-2, respectively) from 2000 to 2006. One plot with about 40 plants in two 1-m long rows for each DH line was visually evaluated for the incidence of virus symptoms in March/April and then a pooled leaf sample (i.e., leaves from 10 plants per line) was tested using double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) according to Clark and Adams (1977) with polyclonal BaMMV- and BaYMV-specific antibodies produced by the Serum Bank of the Institute of Epidemiology and Pathogen Diagnostics (JKI, Quedlinburg, Germany). Furthermore, F_1 plants derived from crossings of 'HOR4224' to 'Carola' and 'Express' (*rym4*), to 'Tokyo' (*rym5*), to 'Ea52' (*rym3*) and to 'Chikurin Ibaraki 1' (*rym15* and another bymovirus resistance gene on chromosome 5HS) and parental lines 'HOR4224' and 'HOR10714' were assessed in Lower Saxony in 2009/2010, on fields at Lenglern naturally highly infested with BaYMV-2 and weakly infested with BaMMV. The F_1 plants cultivated in the field were ELISA tested as pooled leaf samples in March.

The same population of 107 DH lines was also tested for resistance to BaMMV-ASL1 in a growth chamber, following a mechanical virus inoculation procedure. In this test, 6 plants of each DH line were inoculated at the 2- to 3-leaf stage two times at an interval of 5–7 days using sap extracted from the leaves of infected barley plants by homogenization in 0.1 M K_2HPO_4 buffer pH 9.8. Approximately, 5 ml of buffer was used for each 1 g of infected leaf material. To aid mechanical inoculation, 0.1 g of carborundum (mesh 400) was added per 5 ml sap. After inoculation, plants were cultivated in a growth chamber at 12/10 °C day/night and 16-h photoperiod (illuminance 10 kLx). Five weeks after the first inoculation, the plants were assessed by DAS-ELISA for the presence of BaMMV as described above. In the same way, F_1 plants were tested for their reaction to BaMMV-ASL1. Furthermore, the four cultivars carrying different resistance conferring alleles of *Hv-eIF4E* were also tested for their reaction to the three sequenced isolates of BaMMV: BaMMV-ASL1 (Timpe and Kuehne 1994, 1995), BaMMV-Sil (Kanyuka et al. 2004) and BaMMV-Teik (Habekuss et al. 2008) in a growth chamber investigating 10 plants per cultivar.

DNA extraction and molecular mapping

Leaf tissue of each genotype was harvested, freeze-dried and ground to a fine powder for DNA extraction. DNA was extracted essentially as described by Graner et al. (1991). Four DNA bulks, two for each virus, were created by combining equal amounts of DNA from ten DH lines each displaying the same reaction to a particular virus species for bulked segregant analysis (Michelmore et al. 1991). Seven microsatellites (SSRs) for each of the seven barley chromosomes (i.e., 49 microsatellites in total) were investigated to assign resistance genes to chromosomes (Supplementary table 1). PCR amplifications were performed in a GeneAmpSystem 9700 (Perkin Elmer). Reactions and cycling conditions for SSRs and additional size polymorphism markers were as described in Becker and Heun (1995), Liu et al. (1996), Ramsay et al. (2000) and Stein et al. (2005). Routinely, PCR was done in 10 µl reaction volumes containing 20 ng genomic DNA, 0.5 U of Taq DNA Polymerase (Solis Biodyne, Tartu, Estonia), 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 0.2 µM of each primer according to Perovic et al. (2013). Furthermore, the DNA bulks were subjected to AFLP analyses using 144 primer combinations. The restriction enzymes used in preparation of AFLP templates were *Eco*RI and *Mse*I. A first pre-amplification was carried out with *Eco*RI + 0 and *Mse*I + 0 primers. Primers with one additional 3′ terminal nucleotide (*Eco*RI primer + A and *Mse*I primer + C or + A) were used for the second pre-amplification, whereas primers with three additional 3′ terminal nucleotides were used for selective amplifications. AFLP analyses were conducted using the GIBCO BRL AFLP Core Reagent Kit following manufacturer's protocols. Amplified fragments were separated on an ALF Express Genetic Analyser (Amersham Biosciences, Freiburg, Germany) using ReproGel High Resolution polyacrylamide gels (Amersham Biosciences, Freiburg, Germany).

The resistance data of DH population plants were subjected to Chi-squared (χ^2) analysis to determine the goodness-of-fit of observed ratios to theoretical expectations. Genetic maps were constructed by JoinMap 4.0 (van Ooijen 2006) using the regression algorithm and applying the Kosambi function (Kosambi 1944). Only markers with an LOD score of 3 were integrated into the map.

RNA extraction, FlcDNA synthesis and sequencing

Total RNA was extracted from barley leaves using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Subsequently, a DNase I, RNase-free (Fermentas, Germany) digest, was performed according to the manufacturer's instructions. First strand cDNA was synthesized with 1.2 μg total RNA and 0.5 μg oligo(dT)18 primers using the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, Germany). cDNA quantity and quality were evaluated using ND-1000 spectrophotometer (NanoDrop, USA) measurement.

Gene-specific primers for *Hv-eIF4E* were designed based on the published mRNA and the genomic sequence AY661558 (Stein et al. 2005). Full length *Hv-eIF4E* sequences were amplified by RT-PCR with gene-specific primers [HveIF4Es (5-CGGCGCCGCAGGGATGG-3′) and HveIF4Eas (5′-TCGCGGGCCTGCCTTGGAAGC-3′)]. PCR amplification was conducted in 20-μl reaction volume, while purification, sequencing of amplicons, and sequence analysis were conducted as described in Perovic et al. (2013). Purified amplicons were subjected to Sanger cycle-sequencing on a MegaBACE DNA Analysis System (Amersham Biosciences, Freiburg, Germany) and obtained sequences were handled and analyzed utilizing the software packages Sequencher v3.0 (Gene Codes Corporation, Ann Arbor, USA) and BioEdit v7.0 (Hall 1999).

Results

Disease scoring

Visual evaluation and DAS-ELISA-based disease scoring of 107 DH lines during seven growing seasons at three locations (i.e., one field in Eikeloh and two fields in Aschersleben) in combination with mechanically BaMMV-inoculated plants in a growth chamber yielded a uniform and consistent dataset. In general, four different phenotypic classes were observed as follows: 21 DH lines were resistant to BaMMV only, 22 DH lines were resistant to both BaYMV-1 and BaYMV-2, 28 DH lines were completely susceptible and 36 DH lines were resistant to BaMMV, BaYMV-1 and BaYMV-2. The χ^2 test determined that the ratio 21:22:28:36 fitted well to the expected segregation ratio 1:1:1:1 ($\chi^2_{1:1:1:1} = 5.58$, $p = 0.134$) (Table 1), indicating that two independent genes control the resistance to BaMMV and BaYMV in 'HOR4224'. Further detailed analysis revealed that DH lines segregated for BaMMV resistance in a ratio of 57 resistant (*R*) to 50 susceptible (S) ($\chi^2_{1:1} = 0.477$, $p = 0.49$), which fits well to a theoretical expectation of 1R:1S. DH lines segregated for BaYMV resistance in a ratio of 58 resistant to 49 susceptible $\chi^2_{1:1} = 0.77$, $p = 0.38$), which also fits well to a theoretical expectation of 1R:1S. These analyses, therefore, indicated a monogenic inheritance for resistance to both viruses encoded by independent loci.

Table 1 Classification of the DH mapping population based on the reaction to the different viruses of the barley yellow mosaic virus complex

Phenotypic class	BaMMV-ASL1	BaYMV-1	BaYMV-2	Number of DH lines
I	+	−	−	21
II	−	+	+	22
III	+	+	+	36
IV	−	−	−	28
Total				107

+ resistant, − susceptible

Genetic mapping and marker saturation

BaYMV

Out of the 49 SSR markers (Supplementary table 1), equally distributed over the barley genome, three microsatellites (Bmac096, Bmag337 and HVM30) located on the short arm of chromosome 5H were polymorphic between the DNA bulks from DH lines susceptible to BaYMV-1/BaYMV-2 and the DNA bulk from DH lines resistant to BaYMV-1/BaYMV-2. Additional SSRs located on the short arm of chromosome 5H were screened for polymorphism and linkage was detected for Bmag005, Bmac113, HVACL1, Bmag387 and Bmac303 (Table 2; Fig. 1). By this approach, resistance to BaYMV-1/BaYMV-2 was mapped to chromosome 5HS. In addition to SSRs, AFLPs were applied to develop more closely linked markers. Altogether eight microsatellites and 21 AFLP markers linked to the resistance locus on 5H were identified. Microsatellite loci Bmac096 and Bmac303 flanked the resistance locus at a distance of approximately 1 cM.

BaMMV

In parallel, the DNA bulk from DH lines susceptible to BaMMV and the DNA bulk from DH lines resistant to BaMMV were analyzed with the selected markers to

Table 2 Size of alleles detected in the resistant 'HOR4224' and susceptible 'HOR10714' parents for polymorphic markers located on chromosomes 3HL and 5HS of barley

Marker name	Chromosome	Allele Size in bp		References
		HOR4224	HOR10714	
Bmag005	5HS	197	191	Ramsay et al. (2000)
Bmac113	5HS	208	218	Ramsay et al. (2000)
HVACL1	5HS	157	168	Becker and Heun (1995)
Bmag387	5HS	119	130	Ramsay et al. (2000)
Bmac303	5HS	145/153	143/161	Ramsay et al. (2000)
Bmac096	5HS	193	209	Ramsay et al. (2000)
Bmag337	5HS	151	146	Ramsay et al. (2000)
HVM30	5HS	171	167	Liu et al. (1996)
EBmac541	3HL	134	125	Ramsay et al. (2000)
HVM62	3HL	246	242	Liu et al. (1996)
EBmac708	3HL	135	123	Ramsay et al. (2000)
EBmag705	3HL	152	129	Ramsay et al. (2000)
HVM70	3HL	154	152	Liu et al. (1996)
Hv-eIF4Es317/as1170	3HL	750	853	Stein et al. (2005)
B793c24	3HL	null	508	Stein et al. (2005)

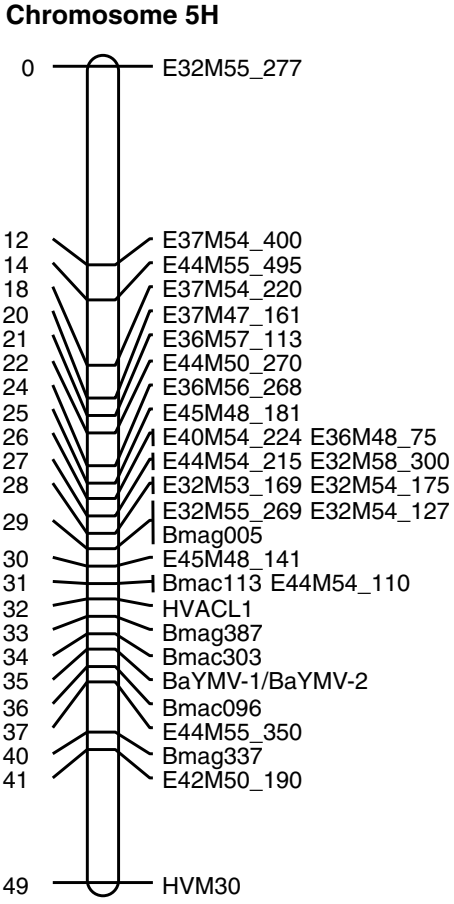

Fig. 1 Partial genetic map of chromosome 5H including the BaYMV-1/BaYMV-2 resistance derived from 'HOR4224'

map the BaMMV resistance locus. Three microsatellites (EBmac0708, EBmac0705 and HVM70) (Table 2) located on the long arm of chromosome 3H were polymorphic between the DNA bulks from DH lines susceptible to BaMMV-ASL1 and the DNA bulk from DH lines resistant to BaMMV-ASL1. As Bmac29 was monomorphic in this cross, additional molecular markers originating from chromosome 3H were selected (data not shown). The INDEL-based marker B793c24 derived from a BAC contig spanning the *Hv-eIF4E* locus (Tyrka et al. 2008) was polymorphic between parental lines and corresponding bulks. Subsequent mapping of this marker in the DH population indicated co-segregation with the BaMMV (BaMMV-ASL1) resistance and suggested that the BaMMV resistance gene in 'HOR4224' is located in the same chromosomal region as *rym4/rym5* (Fig. 2a). Therefore, the primer pair Hv-eIF4E s317/as1170 (Stein et al. 2005), which amplifies the first exon and intron of *Hv-eIF4E* and reveals a size polymorphism between 'HOR4224' and 'HOR10714' and between the two DNA bulks, was used in a next step for genotyping the entire DH population. No recombination events were detected between *Hv-eIF4E* and the BaMMV resistance in 'HOR4224' (Fig. 2b). Therefore, results give hint that a gene allelic to *Hv-eIF4E* controls BaMMV resistance in 'HOR4224' originating from Japan.

Resistance pattern

The four barley cultivars carrying *Hv-eIF4E*-based resistance and the two parental accessions displayed very

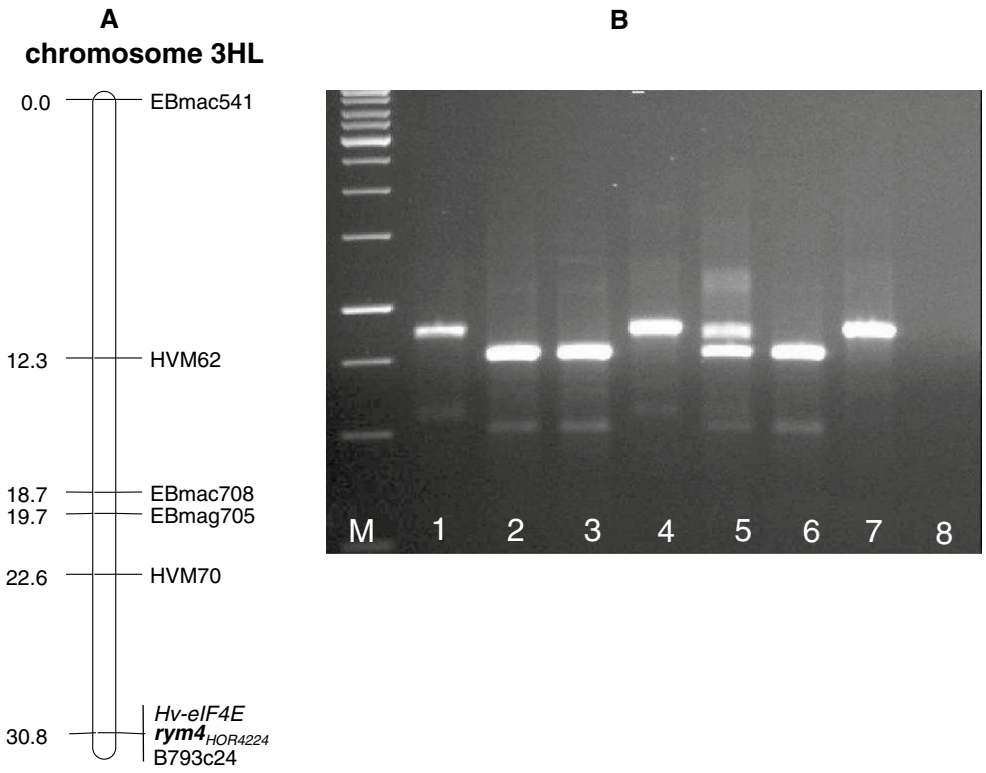

Fig. 2 **a** Partial genetic map of chromosome 3H including the BaMMV resistance derived from 'HOR4224'. **b** Molecular analysis of selected barley genotypes with the *Hv-eIF4E*-based marker s317/as1170. Gel electrophoresis of the *Hv-eIF4E*-specific marker tested on the resistant parental line 'HOR4224' and the susceptible 'HOR10714' resistant and susceptible DH lines and the F_1 plant. The order of genotypes is: *M* 1 kb ladder, *1* 'HOR4224', *2* 'HOR10714', *3* and *6* resistant DHs, *4* and *7* susceptible DHs, *5* F_1 plant, *8* water control

Table 3 Reaction of genotypes carrying different alleles at the Hv-eIF4E locus against five virus strains of the barley yellow mosaic complex

Genotype	Gene	Virus strain				
		BaMMV-Teik	BaMMV-ASL1	BaMMV-Sil	BaYMV-1	BaYMV-2
Franka	*rym4*	r	r	r	r	s
Tokyo	*rym5*	s	r	s	r	r
Miho Golden	*rym6*	(r)*	s	s	r	r
Hiberna	*rym10*	s	s	s	r	r
HOR4224	$rym_{HOR4224}$	r	r	r	r	r
HOR10714	susceptible	s	s	s	s	s

*1 infected plant out of 10 inoculated plants in climatic chamber

different reaction patterns in response to a set of five bymovirus isolates (BaMMV-Sil, BaMMV-ASL1, BaMMV-Teik, BaYMV-1 and BaYMV-2) (Table 3). Cultivar 'Franka' carrying *rym4* showed susceptibility to BaYMV-2, while cultivar 'Tokyo' carrying *rym5* showed susceptibility to two BaMMV isolates BaMMV-Sil and BaMMV-Teik. Cultivar 'Hiberna', the donor of *rym10,* was susceptible to three BaMMV isolates and resistant to both BaYMV isolates, while cultivar 'Miho Golden', a *rym6* donor, displayed resistance to BaMMV-Teik, BaYMV-1 and BaYMV-2, and susceptibility to BaMMV-ASL1 and BaMMV-Sil. 'HOR4244' displayed resistance to all five isolates, while 'HOR10714' displayed susceptibility to all five isolates.

Allelism tests

To determine relatedness between the resistance genes in 'HOR4224' and previously characterized genes conferring resistance to the barley yellow mosaic virus complex, which map to similar chromosomal positions,

Table 4 Allelism test of the BaMMV resistance conferring gene from 'HOR4224'

Genotype/cross	Resistance genes against BaMMV/BaYMV	Chromosomal position	Growth chamber data		Field data (Lenglern)[a]		
			Number of plants tested	BaMMV-ASL1	Extinction BaMMV	Extinction BaYMV	Reaction to BaMMV/BaYMV-2
HOR4224		3HL	10	0/10	0.03	0.04	r/r
Carola	*rym4*	3HL	10	0/10	0.05	1.71	r/s
Carola × HOR4224	F_1		31	0/31	0.04	1.84	r/s
HOR4224 × Carola	F_1		15	0/15	–	–	r/s
Express	*rym4*	3HL	3	0/3	0.05	1.82	r/s
HOR4224 × express	F_1		nt	nt	0.04	2.01	r/s
Tokyo	*rym5*	3HL	nt	nt	0.03	0.02	r/r
Tokyo × HOR4224	F_1		nt	nt	0.05	0.25	r/s

[a] Very weak BaMMV infestation in the field at Lenglern

nt not tested

Table 5 Allele diversity identified for five SSR markers linked to BaYMV resistance at the chromosome 5H on eight barley genotypes

Genotype	Chromosomal position	Reaction to BaYMV-1/BaYMV-2	Marker				
			HVACL1	Bmag387	Bmac303	Bmac096	Bmag337
HOR4224	3HL M[a]/5HS Y[b]	r/r	138	119	146/152	194	150
Ea52	5HS Y	r/r	131/138	119	146/152	194	150
Chikurin Ibaraki 1	6HS M[a]/5HS Y	r/r	140	132	144/152	194	144
Carola	3HL M	r/s	140	140	160	194	167
Tokyo	3HL M	r/r	140	140	158/164	194	167
Express	3HL M	r/s	140	140	160	194	148
HOR10714	–	s/s	149	130	144/161	209	146
Igri	–	s/s	140	140	158/162	194	148

[a] BaMMV, [b] BaYMV

corresponding resistance donors were inter-crossed and tested for resistance (Table 4). DAS-ELISA carried out on BaMMV-ASL1 mechanically infected F_1 plants derived from crosses to carriers of *rym4/rym5* was resistant indicating that the BaMMV resistance gene in 'HOR4224' is allelic to *rym4/rym5*. The F_1 plants of test crosses to 'Carola' (*rym4*), 'Express' (*rym4*) and to 'Tokyo' (*rym5*) were susceptible to BaYMV-2 confirming the results of genetic mapping. F_1 plants obtained from crosses of 'HOR4224' to 'Ea52' (*rym3*) and to 'Chikurin Ibaraki 1' (*rym15* and an unnamed resistance gene on 5HS) were tested by DAS-ELISA for the presence of BaYMV-2 as pooled leaf samples. High extinction values of DAS-ELISA were detected for pooled leaves of 5 and 3 F_1 plants from test crosses to 'Ea52' and 'Chikurin Ibaraki 1', respectively, collected in March. Curiously, leaves sampled from the same F_1 plants at the end of April the same year were tested negative for BaYMV in DAS-ELISA. Since contradictorily DAS-ELISA results have been obtained for the same plant material, it was impossible to draw any conclusion from this allelism test experiment, which will need to be repeated in the future.

Allele diversity of SSR markers linked to the BaYMV resistance on chromosome 5HS

To assess the diagnostic value of the closest linked markers to the BaYMV resistance gene in 'HOR4224' located on the chromosome 5HS and to get insight into the origin of two previously characterized virus resistance genes from the same chromosome, a set of 8 barley accessions was genotyped using five closely linked SSR markers (Table 5). The six selected barley accessions are donors of the BaYMV resistance genes from chromosomes 5HS and 3HL, while 'Igri' and 'HOR10714' are known to be fully susceptible to both BaMMV and BaYMV. Very similar patterns of the examined SSRs were observed between 'HOR4224' and 'Ea52', the *rym3* donor, suggesting the

A

Accession / na position	170	353	359	478	481	614	617	623
HOR4224_r	T	T	C	A	C	C	G	C
HOR10714_sus.	C	A	C	A	C	C	A	G
Franka_*rym4*	T	C	C	A	C	T	G	C
Tokyo_*rym5*	C	A	G	G	A	C	A	G
Miho Golde_*rym6*	C	A	C	A	C	C	A	G
Hiberna_*rym10*	C	A	C	A	C	C	A	G

B

Accession / aa position	57	118	121	161	162	206	207	209
HOR4224_r	F	I	T	N	Q	S	G	A
HOR10714_sus.	S	K	T	N	Q	S	D	G
Franka_*rym4*	F	T	T	N	Q	F	G	A
Tokyo_*rym5*	S	K	S	D	K	S	D	G
Miho Golde_*rym6*	S	K	T	N	K	S	D	G
Hiberna_*rym10*	S	K	T	N	H	S	D	G

Fig. 3 A schematic display of multiple alignment of *Hv-eIF4E* for six cultivars. **a** Display of nucleic acid alignment. **b** Display of amino acid alignment

same or a closely related origin of these two resistance genes. In contrast, the SSR patterns for 'Chikurin Ibaraki 1', the genotypes carrying resistance genes located on chromosomes other than 5HS, and susceptible cultivars were very different from those obtained for 'HOR4224' and 'Ea52'. So, these markers may be well suited for marker-assisted selection.

Hv-eIF4E re-sequencing

The entire open reading frame (ORF) of *Hv-eIF4E* of 648 bp (Fig. 3a) from the parental lines of the DH mapping population, i.e., 'HOR4224' and 'HOR10714', was amplified, sequenced and used in further analysis for the identification of single-nucleotide polymorphisms (SNPs) as well as for comparison to previously characterized allelic variants of the gene. Alignment of sequences from the parental lines revealed four SNPs at positions 170-, 353-, 617- and 623-bp. The coding sequence encodes a protein of 216 amino acid residues, which were used for alignment construction (Fig. 3b). All four SNPs are missense mutations by changing the properties of the involved amino acid residues significantly. Comparative analysis of alignments to data obtained by Stracke et al. (2007) and Hofinger et al. (2011) revealed that one of the haplotypes (#25; 'Aizu 6') carries the T353 SNP and an identical sequence. However, no information on the resistance against European strains of BaMMV/BaYMV is available for this accession. Furthermore, Stein et al. (2005) identified the same haplotype as in 'HOR4224' in four barley accessions, i.e., Yukishirazu, Iwate Mensury 2, Hosomugi 3 and Kenyoshi 1. Based on data reported by Götz and Friedt (1993) on the resistance of these cultivars and due to missing segregation analyses for both viruses, they came to the conclusion that this allele is effective against BaMMV and BaYMV. However, the results of the segregation analyses obtained in this study clearly show that this allele is only effective against BaMMV and that this is due to the T353 SNP as the rest of the coding sequence of HOR4224 is the same as that of *rym4*. In summary, biological data of the test for allelism and segregation analyses and sequence information obtained for *Hv-eIF4E* have shown that 'HOR4224' carries an allele of *Hv-eIFE*, which is exclusively effective against BaMMV, but at the same time encodes susceptibility against BaYMV isolates.

Discussion

Since the gene pool of cultivated barley is largely depleted for major resistance genes for many plant pathogens and many new soil-borne virus isolates have been identified (Nomura et al. 1996; Hariri et al. 2000; Habekuß et al. 2009; Kastirr et al. 2012), there is an obvious need to broaden the genetic base of resistance in modern barley cultivars. Furthermore, the resistance breeding to soil-borne viruses, which is a prerequisite for the cultivation of winter barley in infested areas, relies on the identification of resistant germplasm, the chromosomal localization of corresponding genes and the development of molecular markers that could accelerate the incorporation of new resistance genes in elite lines. In this regard, in the past, e.g., (Proeseler et al. 1989; Kawada 1991; Ordon et al. 1993) large screening programs aiming at the identification of new sources of resistance were launched. Upon identification of different resistance sources, mapping of novel resistance genes is an essential step towards the exploitation of these resources in breeding programs, but it also builds a way towards map-based gene cloning (Palloix and Ordon 2011).

Previous screening of barley accessions from the IPK Gene Bank led to the identification of the accession 'HOR4224' carrying resistance to three strains of *Barley mild mosaic virus* (BaMMV-ASL1, BaMMV-Sil and BaMMV-Teik) and to two strains of *Barley yellow mosaic virus* (BaYMV-1 and BaYMV-2). The resistance pattern of 'HOR4224' in response to five isolates of bymoviruses was very different to the resistance patterns of the cultivars carrying resistance genes *rym4*, *rym5*, *rym6* or *rym10* (Table 3). Genetic analysis of the DH population derived from a cross between 'HOR4224' and a susceptible barley accession 'HOR10714' indicated the independent inheritance of resistance to BaMMV and BaYMV controlled by a single gene, each, as this has also been shown in Chikurin Ibaraki I (Werner et al. 2003, Le Gouis et al. 2004). Subsequent mapping positioned a gene for resistance to BaMMV to chromosome 3HL, and a gene for resistance to BaYMV to chromosome 5HS. Several previously characterized bymovirus resistance genes are known to reside in these chromosomal regions: *rym4*, *rym5*, *rym6* and *rym10* on

3HL, and *rym3* and an unnamed resistance gene from cv. 'Chikurin Ibaraki 1' on 5HS (Werner et al. 2003; McGrann and Adams 2000).

The BaMMV resistance in 'HOR4224' was mapped to the chromosome 3HL region carrying the *rym4*/*rym5* locus (Fig. 2A), which is known to correspond to *Hv-eIF4E* (Kanyuka et al. 2005; Stein et al. 2005). An allelism test confirmed that the gene for resistance to BaMMV in 'HOR4224' is allelic to *rym4*/*rym5* (Table 4). Sequencing of the coding region of *Hv-eIF4E* revealed a novel allele of this gene in 'HOR4224' (Fig. 3), which confers resistance to isolates of BaMMV in fields (Eikeloh and Aschersleben) and the mechanical transmitted isolate BaMMV-ASL1, but is ineffective against BaYMV-1 or BaYMV-2 (Table 3). One of the non-synonymous amino acid substitutions (I118K) identified in *Hv-eIF4E* of 'HOR4224' and of five previously detected genotypes (Hofinger et al. 2011 and Stein et al. 2005) appears to be a unique feature and the main difference between *rym4* and 'HOR4224' (Fig. 3b). Due to a similarity of resistance patterns, sequence similarity and confirmed allelism, we propose to name this new resistance allele of the *Hv-eIF4E* as $rym4_{HOR4224}$. This result, at the first instance, confirmed the power to detect new alleles if the gene of interest is previously isolated and from the breeding point of view, enabled the development of two molecular markers for use in crossing schemes, an INDEL-based marker Hv-eIF4Es317/as1170 (Table 2; Fig. 2b) and an SNP-based marker at the position 353-bp.

Regarding mapping of BaYMV resistance, the gene conferring resistance to BaYMV-1 and BaYMV-2 in the Japanese cultivar 'Chikurin Ibaraki 1' has been mapped in the same region of chromosome 5HS (Werner et al. 2003) as well as the resistance gene *rym3* of 'Ea 52' (Saeki et al. 1999), which is a gamma ray-induced mutant of 'Chikurin Ibaraki 1' (Ukai 1984). In this respect, it is interesting to notice that 'Chikurin Ibaraki 1' is susceptible to BaYMV in Japan (Ukai and Yamashita 1980) but completely resistant to BaMMV, BaYMV-1 and BaYMV-2 in Germany (Götz and Friedt 1993). This is confirmed by this study in which 'Chikurin Ibaraki 1' was completely resistant to BaYMV-1, BaYMV-2 and BaMMV in the field and to BaMMV-ASL1 and BaMMV-Teik in the growth chamber. At the same time, accession 'Ea 52' is only resistant to BaYMV-1 and BaYMV-2 (Götz and Friedt 1993). The BaMMV resistance of 'Chikurin Ibaraki 1' has been mapped on chromosome 6H (Le Gouis et al. 2004). Comparison of genetic maps of three BaYMV resistance genes from chromosome 5HS does not answer the question whether we are dealing with one gene having various alleles or it is a case of independent closely linked genes. Assessment of allelic diversity of closely linked SSRs (Table 5) provides a hint of close relatedness of the *rym3* donor 'Ea52' and 'HOR4224', while 'Chikurin Ibaraki 1' turned out to be different. Nevertheless, further tests for allelism are needed to unequivocally answer the question whether *rym3*, and the BaYMV resistance gene in 'HOR4224' and 'Chikurin Ibaraki 1' are allelic or represent independent closely linked genes.

Closely linked markers, which are available for many resistance genes against the barley yellow mosaic virus complex (Ordon and Perovic 2013), facilitate efficient marker-based selection procedures and allow pyramiding of resistance genes which may lead to more durable resistances (Werner et al. 2005). In this respect, markers identified in this study could be used for direct transfer of resistances into elite barley lines (Ordon et al. 2009). Moreover, the BaYMV resistance markers identified in this work can be used as starting points towards positional isolation of the corresponding resistance gene (Pellio et al. 2005, Lüpken et al. 2013). Results of this study on the one hand demonstrate the power of integrated mapping and targeted allele mining with respect to broadening the genetic base of resistance, while on the other hand the identified novel allele of the *rym4* gene may contribute to a further understanding of virus-host plant interaction.

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Ethical standards All experiments and data analysis have been performed according to ethical standards.

References

Adams MJ, Swaby AG, Jones P (1988) Confirmation of the transmission of barley yellow mosaic virus (BaYMV) by the fungus *Polymyxa graminis*. Ann Appl Biol 112:133–141

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 27:835–845

Boisnard A, Albar L, Thiéméle D, Rondeau M, Ghesquière A (2007) Evaluation of genes from *eIF4E* and *eIF4G* multigenic families as potential candidates for partial resistance QTLs to *Rice yellow mottle virus* in rice. Theor Appl Genet 116(1):53–62

Clark MF, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34:475–483

Fraser RSS (1990) The genetics of resistance to plant viruses. Annu Rev Phytopathol 28:179–200

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, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map in 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 fine structure of the *rym5* locus encoding resistance to different strains of the Barley Yellow Mosaic Virus Complex. Theor Appl Genet 98:285–290

Habekuß A, Krämer I, Proeseler G, Pickering R (2000) Genetic and molecular characterization of virus resistance in winter barley. Czech J Genet Plant Breed 36:79–83

Habekuss A, Kühne T, Krämer I, Rabenstein F, Ehrig F, Ruge-Wehling B, Huth W, Ordon F (2008) Identification of barley mild mosaic virus isolates in Germany breaking *rym5* resistance. J Phytopathol 156:36–41

Habekuß A, Riedel C, Schliephake E, Ordon F (2009) Breeding for resistance to insect-transmitted viruses in barley—an emerging challenge due to global warming. J für Kulturpflanzen 61:53–61

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows. Nucl Acids Symp Ser 41:95–98

Hariri D, Meyer M, Le Gouis J, Bahrman N, Fouchard M, Forget C, Andre A (2000) Characterisation of BaYMV and BaMMVpathotypes 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

Hofinger BJ, Jing H-C, Hammond-Kosack KE, Kanyuka K (2009) High-resolution melting analysis of cDNA-derived PCR amplicons for rapid and cost-effective identification of novel alleles in barley. Theor Appl Genet 119:851–865

Hofinger BJ, Russell JR, Bass CG, Baldwin T, Dos Reis M, Hedley PE, Li Y, Macaulay M, Waugh R, Hammond-Kosack KE, Kanyuka K (2011) An exceptionally high nucleotide and haplotype diversity and a signature of positive selection for the *eIF4E* resistance gene in barley are revealed by allele mining and phylogenetic analyses of natural populations. Mol Ecol 20:3653–3668

Kai H, Takata K, Tsukazaki M, Furusho M, Baba T (2012) Molecular mapping of *Rym17*, a dominant and *rym18* a recessive *Barley yellow mosaic virus* (BaYMV) resistance genes derived from *Hordeum vulgare* L. Theor Appl Genet 124:577–583

Kanyuka K, McGrann G, Alhudaib K, Hariri D, Adams MJ (2004) Biological and sequence analysis of a novel European isolate of Barley mild mosaic virus that overcomes the barley *rym5* resistance gene. Arch Virol 149:1469–1480

Kanyuka K, Druka A, Caldwell DG, Tymon A, McCallum N, Waugh R, Adams MJ (2005) Evidence that the recessive bymovirus locus *rym4* in barley corresponds to the eukaryotic translation initiation factor 4E gene. Mol Plant Pathol 6:449–458

Kastirr U, Ziegler A, Perovic D, Golecki B (2012) Occurrence of soil-borne wheat mosaic virus (SBWMV-NY) in Schleswig-Holstein and its importance for wheat cultivation. J für Kulturpflanzen 64:469–477

Kaur N, Kenneth S, Mackay M, Yahiaoui N, Keller B (2008) Molecular approaches for characterization and use of natural disease resistance in wheat. Eur J Plant Pathol 121:387–397

Kawada N (1991) Resistant cultivars and genetic ancestry of the resistance genes to *Barley yellow mosaic virus* in barley (*Hordeum vulgare* L.). Bull Kyushu Natl Agric Exp Sta 27:65–79

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

Kühne T (2009) Soil-borne viruses affecting cereals: known for long but still a threat. Virus Res 141:174–183

Le Gouis J, Devaux P, Werner K, Hariri D, Bahrman N, Béghin D, Ordon F (2004) *rym15* from the Japanese cultivar Chikurin Ibaraki 1 is a new *Barley mild mosaic virus* (BaMMV) resistance gene mapped on chromosome 6H. Theor Appl Genet 108:1521–1525

Ling KS, Harris KR, Meyer JDF, Levi A, Guner N, Wehner TC, Bendahmane A, Havey MJ (2009) Non-synonymous single nucleotide polymorphisms in the watermelon eIF4E gene are closely associated with resistance to Zucchini yellow mosaic virus. Theor Appl Genet 120:191–200

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

Lopez-Moya JJ, Garcia JA (2008) Construction of a stable and highly infectious intron-containing cDNA clone of plum pox potyvirus and its use to infect plants by particle bombardment. Virus Res 68:99–107

Lüpken T, Stein N, Perovic D, Habekuß A, Krämer I, Hähnel U, Steuernagel B, Scholz U, Zhou R, Ariyadasa R, Taudien S, Platzer M, Martis M, Mayer K, Friedt W, Ordon F (2013) Genomics-based high-resolution mapping of the BaMMV/BaYMV resistance gene *rym11* in barley (*Hordeum vulgare* L.). Theor Appl Genet 126:1201–1212

Maule AJ, Caranta C, Boulton MI (2007) Sources of natural resistance to plant viruses: status and prospects. Mol Plant Pathol 8:223–231

McCouch S (2004) Diversifying selection in plant breeding. PLoS Biol 2(10):e347

McGrann GRD, Adams MJ (2000) Investigating resistance to *Barley mild mosaic virus*. Plant Pathol 53:161–169

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

Miedaner T, Korzun V (2012) Marker-assisted selection for disease resistance in wheat and barley breeding. Phytopathol 102:560–566

Nomura K, Kashiwazaki S, Hibino H, Inoue T, Nakata E, Tsuzaki Y, Okuyama S (1996) Biological and serological properties of strains of *Barley mild mosaic virus*. J Phytopathol 144(2):103–107

Ordon F, Perovic D (2013) Virus resistance in barley. In: Varshney RK, Tuberosa R (eds) Translational genomics for crop breeding, vol 1: Biotic Stresses, 1st edn. Wiley, pp 63–75

Ordon F, Götz R, Friedt W (1993) Genetic stocks resistant to barley yellow mosaic viruses (BaMMV, BaYMV, BaYMV-2) in Germany. Barley Genet Newsl 22:46–49

Ordon F, Habekuss A, Kastirr U, Rabenstein F, Kühne T (2009) Virus resistance in cereals: sources of resistance, genetics and breeding. J Phytopathol 157:535–545

Palloix A, Ordon F (2011) Advanced breeding for virus resistance in plants. In: Caranta C, Aranda MA, Tepfer M, Moya JJL (eds) Recent advances in plant virology. Caister Academic Press, Norfolk, pp 198–214

Pellio B, Streng S, Bauer E, Stein N, Perovic D, Schiemann A, Friedt W, Ordon F, Graner A (2005) High-resolution mapping of the *Rym4/Rym5* locus conferring resistance to the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2) in barley (*Hordeum vulgare* ssp. *vulgare* L.). Theor Appl Genet 110:283–293

Perovic D, Tiffin P, Douchkov D, Bäumlein H, Graner A (2007) An integrated approach for the comparative analysis of a multigene family: the nicotianamine synthase genes of barley. Funct Integr Genomics 7:169–179

Perovic J, Silvar C, Koenig J, Stein N, Perovic D, Ordon F (2013) A versatile fluorescence-based multiplexing assay for CAPS genotyping on capillary electrophoresis systems. Mol Breed 32:61–69

Pickering RA, Devaux P (1992) Haploid production: approaches and use in plant breeding. In: Shewry PR (ed) Barley: genetics,

biochemistry, molecular biology and biotechnology. CAB International, Wallingford, pp 519–547

Proeseler G, Hartleb H, Kopahnke D, Lehmann CO (1989) Resistenzeigenschaften im Gersten- und Weizensortiment Gatersleben. 27. Prüfung von Gersten auf ihr Verhaltengegenüber dem Milden Gerstenmosaik-Virus (*Barley mild mosaic virus*), Gerstengelbmosaik-Virus (*Barley yellow mosaic virus*), *Drechslera teres* (Sacc.) Shoem. und *Puccinia hordei* Otth. Kulturpflanze 37:145–154

Proeseler G, Habekuss A, Kastirr U, Graner A, Hammer K (1999) Resistance evaluation of winter barley to the barley mosaic virus complex and other pathogens—experiences of 15 years. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 106:425–430

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

Revers F, Le Gall O, Candresse T, Maule AJ (1999) New advances in understanding the molecular biology of plant/potyvirus interactions. MPMI 12:367–376

Robaglia C, Caranta C (2006) Translation initiation factors: a weak link in plant RNA virus infection. Trends Plant Sci 11(1):40–45

Saeki K, Miyazaki C, Hirota N, Saito A, Ito K, Konishi T (1999) RFLP mapping of BaYMV resistance gene *rym3* in barley (*Hordeum vulgare*). Theor Appl Genet 99:727–732

Stein N, Perovic D, Kumlehn J, Pellio B, Stracke S, Streng S, Ordon F, Graner A (2005) The eukaryotic translation initiation factor 4E confers multiallelic recessive Bymovirus resistance in *Hordeum vulgare* (L.). Plant J 42:912–922

Stracke S, Presterl T, Stein N, Perovic D, Ordon F, Graner A (2007) Effects of introgression and recombination on haplotype structure and linkage disequilibrium surrounding a locus encoding bymovirus resistance in barley. Genetics 175:805–817

Timpe U, Kuehne T (1994) The complete nucleotide sequence of RNA2 of barley mild mosaic virus (BaMMV). Eur J Plant Pathol 100:233–241

Timpe U, Kuehne T (1995) In vitro transcriptsof a full-length cDNA of a naturally deleted RNA2 of barley mild mosaic virus (BaMMV) replicate in BaMMV-infected plants. J Gen Virol 76:2619–2623

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

Tyrka M, Perovic D, Wardyńska A, Ordon F (2008) A new diagnostic SSR marker for selection of Rym4/Rym5 locus in barley breeding. J Appl Genet 49:127–134

Ukai Y (1984) Genetic analysis of a mutant resistant to *Barley yellow mosaic virus*. Barley Genetic Newsl 14:31–33

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

Van Ooijen JW (2006) JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Werner K, Fried W, Laubach E, Waugh R, Ordon F (2003) 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, Friedt W, Ordon F (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). Mol Breed 16:45–55

Yeam I, Cavatorta JR, Ripoll DR, Kang BC, Jahn MM (2007) Functional dissection of naturally occurring amino acid substitutions in eIF4E that confers recessive potyvirus resistance in plants. Plant Cell 19:2913–2928