Theor Appl Genet (2003) 106:1425–1432
DOI 10.1007/s00122-002-1188-6

K. Werner · W. Friedt · E. Laubach · R. Waugh · F. Ordon

Dissection of resistance to soil-borne yellow-mosaic-inducing viruses of barley (BaMMV, BaYMV, BaYMV-2) in a complex breeders' cross by means of SSRs and simultaneous mapping of BaYMV/BaYMV-2 resistance of var. 'Chikurin Ibaraki 1'

Received: 7 January 2002 / Accepted: 10 September 2002 / Published online: 28 February 2003

Abstract Ninety-three F_1-derived doubled haploid (DH) lines from a complex breeders' cross involving the Japanese genotype 'Chikurin Ibaraki 1', which is resistant to barley mild mosaic virus (BaMMV) and two strains of barley yellow mosaic virus (BaYMV and BaYMV-2), three susceptible varieties ('Hamu', 'Julia' and a breeding line) and cv. 'Carola', which carries *rym4* conferring resistance to BaMMV and BaYMV, were analysed for resistance to BaMMV, BaYMV and BaYMV-2. The DH lines fell into four phenotypic classes. In addition to completely resistant and susceptible genotypes, DHs were observed which were either resistant to BaMMV and BaYMV or to BaYMV and BaYMV-2. For BaMMV and BaYMV-2 resistance, segregation ratios approaching 1r:1s were observed, suggesting the presence of single resistance genes. In contrast, the segregation ratio for BaYMV fits a 3r:1s segregation ratio, suggesting the presence of two independently inherited genes. From the genetic analysis, we conclude that a resistance locus effective against BaYMV and BaYMV-2 originates from Chikurin Ibaraki 1 and segregates independently from the Carola-derived *rym4* resistance that is effective against BaYMV and BaMMV. The BaMMV resistance in Chikurin Ibaraki 1 has probably been lost during population development. This hypothesis was tested using a simple-sequence repeat (SSR) marker (Bmac29) linked to *rym4*. All BaMMV-resistant DH lines supported amplification of the *rym4*-resistance diagnostic allele. To identify the genetic location of the Chikurin Ibaraki 1-derived resistance against BaYMV/BaYMV-2, bulked DNA samples were constructed from the four resistance classes, and bulked segregant analysis was performed using a genome-wide collection of SSRs. Differentiating alleles were observed at two linked SSRs on chromosome 5H. The location of this BaYMV/BaYMV-2 resistance locus was confirmed and further resolved by linkage analysis on the whole population using a total of five linked SSRs.

Keywords *Hordeum vulgare* · Resistance · Barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2) · Simple sequence repeats · Pyramiding

Communicated by P. Langridge

K. Werner · W. Friedt · F. Ordon (✉)
Institute of Crop Science and Plant Breeding I,
Justus-Liebig-University, Heinrich-Buff-Ring 26-32,
35392 Giessen, Germany
e-mail: Frank.Ordon@agrar.uni-giessen.de

E. Laubach
Nordsaat Saatzucht GmbH, Getreidezuchtstation Gudow,
Hofweg 8, 23899 Segrahn, Germany

R. Waugh
Scottish Crop Research Institute, Dundee, Invergowrie,
Scotland, UK

F. Ordon
Institute of Epidemiology and Resistance,
Federal Centre for Breeding Research on Cultivated Plants,
Theodor-Roemer-Weg 4, 06449 Aschersleben, Germany

Introduction

Since its first detection in Germany in 1978 (Huth and Lesemann 1978) barley yellow mosaic virus disease has spread to most of the winter barley growing areas in western Europe and has become a serious threat to winter barley cultivation in these regions. In Germany the disease is actually caused by barley mild mosaic virus (BaMMV) and two strains of barley yellow mosaic virus, i.e. BaYMV and BaYMV-2 (Huth 1989; Huth and Adams 1990). Due to transmission of the viruses by the soil-borne fungus *Polymyxa graminis* (Toyama and Kusaba 1970), chemical measures to prevent the high yield losses frequently observed in susceptible winter barley crops are neither efficient nor economic. Therefore, breeding for resistance against yellow-mosaic-inducing viruses is of special importance to ensure continued winter barley cultivation in an expanding area of infested land. As a result of extensive screening programmes (e.g. Friedt et al. 1985; Proeseler et al. 1989; Huth 1991), resistance has been identified within the primary gene pool of barley,

Table 1 Mapped resistance genes against barley yellow mosaic virus disease, their source, resistance of the donor in Germany, and virus used for mapping

Resistance Gene	Chromosome	Source	Resistance of donor in Germany	Virus used for mapping	Literature[a]
rym1	4HL	Mokusekko 3	BaMMV, BaYMV, BaYMV-2	BaYMV[b]	1, 2, 4, 10
rym2	7HL	Mihori Hadaka 3	BaMMV, BaYMV, BaYMV-2	BaYMV[b]	1, 2, 4
rym3	5HS	Ea 52, Ishuku Shirazu	BaYMV, BaYMV-2	BaYMV[b]	2, 4, 14
rym4	3HL	Ragusa, Franka	BaMMV, BaYMV	BaMMV, BaYMV	2, 3, 4, 7
rym5	3HL	Mokusekko 3, Resistant Ym No. 1, W122/37.1	BaMMV, BaYMV, BaYMV-2	BaMMV, BaYMV, BaYMV-2, BaYMV[b]	2, 4, 6, 10, 11
rym6	3HL	Prior, Amagi Nijo	susceptible	BaYMV[b]	5, 13, 15
rym7	1HS	HHor 3365	BaMMV	BaMMV	12
rym8	4HL	10247	BaMMV, BaYMV	BaMMV	2, 4, 9, 12
rym9	4HL	Bulgarian 347	BaMMV	BaMMV	2, 4, 9
rym10	3HL	Hiberna	BaYMV, BaYMV-2	BaYMV, BaYMV-2	6, 11
rym11	4HL	Russia 57	BaMMV, BaYMV, BaYMV-2	BaMMV	2, 4, 9
rym12	4HL	Muju covered 2	BaMMV, BaYMV, BaYMV-2	BaMMV	2, 4, 8
rym13	4HL	Taihoku A	BaMMV, BaYMV, BaYMV-2	BaMMV	2, 4, 16

[a] 1, Takahashi et al. (1973); 2, Götz and Friedt (1993); 3, Graner and Bauer (1993); 4, Ordon et al. (1993); 5, Iida and Konishi (1994); 6, Graner et al. (1995); 7, Ordon et al. (1995); 8, Graner et al. (1996); 9, Bauer et al. (1997); 10, Konishi et al. (1997); 11, Graner et al. (1999a); 12, Graner et al. (1999b); 13, Iida et al. (1999); 14, Saeki et al. (1999); 15, Konishi et al. (2002); 16, Werner et al. (2003)
[b] Japanese strain of BaYMV

and genotypic differences concerning reaction to the different members of the barley yellow mosaic virus complex have been observed (Ordon et al. 1993).

Currently, the resistance of cultivars released in Europe is mainly based on the recessive resistance gene *rym4*, which is not effective against BaYMV-2. However, *rym5*, derived from the Chinese landrace 'Mokusekko 3' (Konishi et al. 1997; Graner et al. 1999a), additionally confers resistance to BaYMV-2 and has recently been incorporated into adapted cultivars such as 'Tokyo' or 'Kamoto' (Friedt et al. 2000; Anonymous 2001). An overview on mapped resistance genes against barley yellow mosaic virus disease, the resistance of the donor in Germany and the virus or virus strains used for mapping is given in Table 1. Both *rym4* and *rym5* form a complex locus on the long arm of chromosome 3H (Table 1, Graner et al. 1995, 1999a, b) and are allelic with respect to BaMMV (Götz and Friedt 1993). In addition to *rym4* and *rym5*, several other genes conferring resistance against the different yellow-mosaic-inducing viruses have been identified within the barley gene pool (Götz and Friedt 1993; Ordon and Friedt 1993) and have been assigned to barley chromosomes (Table 1, Graner et al. 2000). Resistance gene *rym7*, which confers partial resistance to BaMMV, has been mapped to the centromeric region of chromosome 1HS (Graner et al. 1999b). Resistance gene *rym9*, derived from the var. 'Bulgarian 347' and exclusively effective against BaMMV, was located in the telomeric region of chromosome 4HL, as were *rym8*, which encodes partial resistance (Bauer et al. 1997), *rym12* and *rym13* (Graner et al. 1996; Werner et al. 2003). Resistance gene *rym11* from cv. 'Russia 57' was mapped to the centromeric region of 4HL (Bauer et al. 1997), and *rym4*, *rym5* and *rym10* have been mapped to the telomeric region of chromosome 3HL (Graner et al. 1995, 1999a; Konishi et al. 1997). By using a Japanese strain of BaYMV, *rym1* was located on chromosome 4HL (Takahashi et al. 1973; Konishi et al. 1997), *rym2* of 'Mihori Hadaka 3' on chromosome 7HL (Takahashi et al. 1973) and *rym6* also in the telomeric region of chromosome 3HL (Iida and Konishi 1994; Iida et al. 1999). Saeki et al. (1999) assigned *rym3*, derived from 'Ea52' (Ukai 1984), to chromosome 5HS. Interestingly, Ea52 is a gamma-ray-induced mutant of Chikurin Ibaraki 1, which is susceptible to barley yellow mosaic virus in Japan but resistant to BaMMV, BaYMV and BaYMV-2 in Germany (Götz and Friedt 1993). In contrast, Ea52 and 'Ishuku Shirazu', which have been used to map *rym3* (Saeki et al. 1999), are susceptible to BaMMV but resistant to BaYMV and BaYMV-2 in Europe (Götz and Friedt 1993; Ordon et al. 1993). Due to the fact that Chikurin Ibaraki 1 is resistant against all agents of the barley yellow mosaic virus complex known so far in Europe, it is of special importance for barley breeding. This is especially true as it has been shown by segregation analysis that the BaMMV resistance in Chikurin Ibaraki 1 is not allelic to *rym4* and *rym5* (Götz and Friedt 1993) and is inherited in a monogenic recessive manner (Ordon and Friedt 1993). As a result, attempts have been made to map the resistance of Chikurin Ibaraki 1 and to provide easy-to-handle polymerase chain reaction (PCR)-based markers for it to facilitate marker-based selection to accelerate backcrossing procedures (Ordon et al. 1999) and allow pyramiding of resistance genes (Werner et al. 2000a, b).

In this respect simple-sequence repeats (SSRs) may be considered well-suited markers because of their locus specificity, ease of use, high reproducibility, and co-dominant mode of inheritance (Macaulay et al. 2001). Linkage between SSRs and genes conferring resistance against the barley yellow mosaic virus complex has been detected for *rym9* (Werner et al. 2000a), *rym11* (Bauer et al. 1997) and *rym13* (Werner et al. 2003). Furthermore, the SSR Bmac29, closely linked to the *rym4*/*rym5* locus (Graner et al. 1999a), is already being used extensively in marker-based selection in European barley breeding programmes (Schiemann and Backes 2000).

Because Chikurin Ibaraki 1 is not adapted to European growing conditions (Ordon and Friedt 1994; Ordon et al. 1997) complex breeders crosses have been carried out with Chikurin Ibaraki 1 in barley improvement programmes in the last decade and the resulting DH lines were used for analysing the Chikurin Ibaraki 1 resistance in an adapted genetic background.

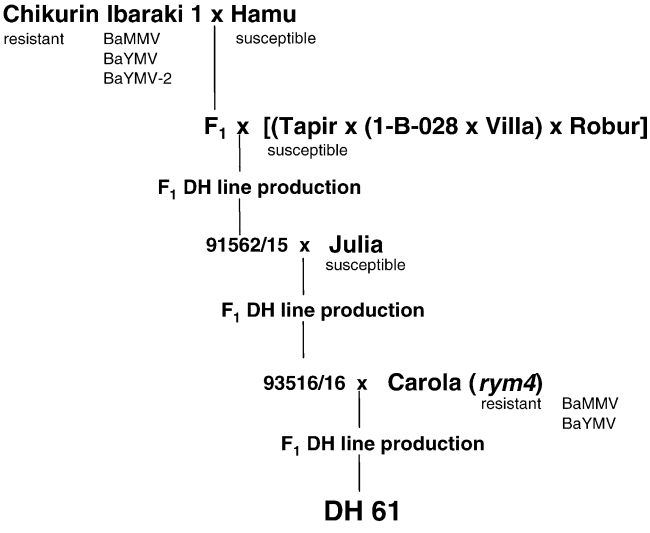

Fig. 1 Pedigree of the mapping population DH 61 and resistance properties of the parents against the barley yellow mosaic virus complex in Germany

Materials and methods

Genetic analysis was carried out on 93 doubled haploid (DH) lines (DH61) derived from a complex cross involving Japanese variety Chikurin Ibaraki 1, which is resistant to BaMMV, BaYMV and BaYMV-2 in Europe (Götz and Friedt 1993). Chikurin Ibaraki 1 was first crossed to susceptible cultivar 'Hamu', and the F_1 was directly crossed to a susceptible breeding line involving cvs. 'Tapir', 'Villa' and 'Robur' and the *Hordeum spontaneum* line 1-B-028. The F_1-derived DH line 91562/15 was crossed to susceptible cultivar 'Julia', and DH-line 93516/16 was crossed to cv. 'Carola'. This cultivar carries *rym4* conferring resistance to BaMMV and BaYMV. Detailed information on the pedigree of this DH population is given in Fig. 1.

Reaction to BaMMV was assessed by mechanical inoculation in the greenhouse according to Friedt (1983) in two replications comprising five plants per DH line followed by DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay; for details see Ordon and Friedt 1993). Since neither BaYMV nor BaYMV-2 can be transmitted mechanically at sufficient infection rates, field experiments were performed in 1999/2000 and in 2000/2001 at four locations – two infested with BaMMV and BaYMV (Giessen Hesse and Höhefeld Bavaria) and two infested with

Table 2 Simple-sequence repeats (SRRs) tested for polymorphism between resistant and suceptible bulks

SSR	Chromosome	Localisation (cM) (Ramsay et al. 2000)	SSR fragment pattern on bulks
Bmac0399	1H	25	Monomorphic
Bmac0032	1H	55	Monomorphic
EBmac0656	1H	63	Monomorphic
WMC1E8	1H	164	Monomorphic
Bmac0134	2H	5	Monomorphic
Bmag0378	2H	44	Monomorphic
HVM54	2H	103	Monomorphic
EBmac0415	2H	105	Monomorphic
HvLTPPB	3H	25	Monomorphic
Bmag0136	3H	50	Monomorphic
Bmag0225	3H	74	Monomorphic
HVM62	3H	154	Monomorphic
HVM40	4H	14	Monomorphic
Bmag0353	4H	45	Monomorphic
EBmac0701	4H	76	Monomorphic
HVM67	4H	118	Monomorphic
Bmac0113	5H	41	Polymorphic
EBmac0970	5H	54	Polymorphic
Bmag0223	5H	69	Monomorphic
Bmag0222	5H	162	Monomorphic
Bmac0316	6H	6	Monomorphic
Bmac0018	6H	103	Monomorphic
Bmag0009	6H	103	Monomorphic
Bmac0040	6H	151	Monomorphic
Bmag0021	7H	13	Monomorphic
HvCMA	7H	85	Monomorphic
Bmag0120	7H	118	Monomorphic
Bmac0156	7H	156	Monomorphic

BaYMV-2 (Eikeloh Northrhine-Westphalia and Schladen Lower Saxony). Each line was tested in these fields in two replications comprising about 30 plants each. Besides visual assessment, the reaction to the different viruses was determined by DAS-ELISA using specific antisera against BaMMV and BaYMV [kindly provided by Dr. Frank Rabenstein, Federal Centre for Breeding Research on Cultivated Plants (BAZ), Aschersleben, Germany]. Optical density was estimated photometrically at a measurement wavelength of 405 nm and a reference wavelength of 620 nm (Easy Reader 400 ATX, SLT-Labinstruments, Crailsheim, Germany).

DNA of each DH line was extracted according to Doyle and Doyle (1990). DNA concentration was determined using a Fluorometer (Hoefer Scientific Instuments) and diluted to a final concentration of 25 ng/μl. Based on the phenotypic data, bulks comprising equal amounts of DNA of eight DH lines each were constructed for bulked segregant analysis (Michelmore et al. 1991). Polymerase chain reactions (PCR) were carried out in a GeneAmp System 9700 (Perkin Elmer, Alameda, Calif.). In order to assign resistance genes to chromosomes four SSRs per chromosome were analysed in a first step (Table 2). Detailed information on primer sequences, amplification and PCR conditions of the respective SSRs are given in Liu et al. (1996) and Ramsay et al. (2000); see also the homepage of the Scottish Crop Research Institute (SCRI), http://www.scri.sari.ac.uk. Bmac29, closely linked to the *rym4/rym5* locus, was assayed according to Graner et al. (1999a). PCR products were separated on an 8% Long Ranger Gel (FMC Biozym, Hessisch Oldendorf, Germany), and fluorescently labelled products were detected on a LI-COR DNA Sequencer Genreadir 4200 (MWG Biotech AG, Ebersberg, Germany).

Linkage analysis was carried out using the MAPMAKER software (Lander et al. 1987). Crossover units were converted into map distances (centiMorgans) by applying the Kosambi function (Kosambi 1944).

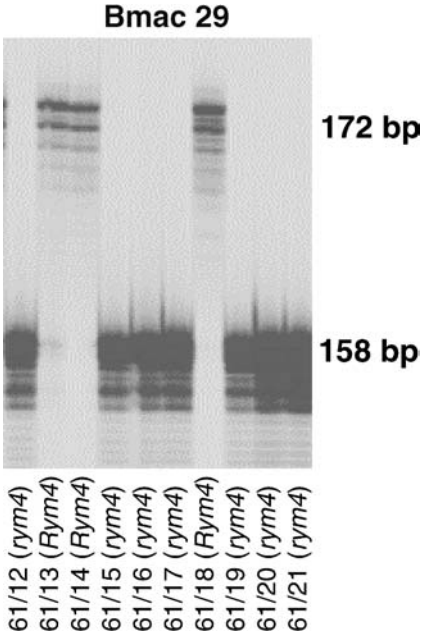

Fig. 2 Fragment pattern of microsatellite Bmac29 in the mapping population on lines being homozygous-recessive (*rym4*) and homozygous-dominant at the *Rym4* locus

Results

Results of the resistance tests concerning BaMMV, BaYMV and BaYMV-2 are shown in Table 3. Four phenotypic classes can be distinguished. In addition to lines which are completely resistant or susceptible to all members of the barley mosaic virus complex, there are lines exclusively resistant either to BaMMV and BaYMV or to BaYMV and BaYMV-2. Segregation for BaYMV-2 (49r:44s) perfectly fits a 1r:1s ($\chi^2 = 0.269$, $0.50 < P < 0.80$) segregation ratio indicative of the presence of a single recessive resistance gene. In contrast, segregation for BaYMV (66r:27s) fits a segregation ratio of 3r:1s ($\chi^2 = 0.806$, $0.20 < P < 0.50$), which is expected in DH populations when two independent genes confer resistance. BaMMV resistance revealed a segregation ratio of 36r:57s ($\chi^2 = 4.742$, $0.01 < P < 0.05$). These results suggest that BaMMV resistance in this DH population is derived exclusively from *rym4*, BaYMV-2 resistance is derived exclusively from Chikurin Ibaraki 1, and BaYMV from *rym4* and Chikurin Ibaraki 1. In order to prove this assumption the population was genotyped with the *Rym4/rym4* diagnostic SSR marker Bmac29 that is tightly linked to the resistance gene *rym4* (Graner et al. 1999a). Genotypic analysis confirmed that all DH lines included in groups 2 and 3 – i.e. resistant to BaMMV (Table 3) – supported amplification of a 158-bp allele which is diagnostic of the presence of the recessive resistance encoding allele *rym4* (Fig. 2). Progeny in groups 1 and 4 – i.e. susceptible to BaMMV – supported amplification of the diagnostic susceptible 172-bp allele (Fig. 2). These results suggest that the resistance of Chikurin Ibaraki 1 to BaYMV and BaYMV-2 is encoded by a single or two very closely linked genes, as all resistant lines were resistant to both strains.

In order to localise the BaYMV/BaYMV-2 resistance of Chikurin Ibaraki 1 onto the barley genetic map, we assembled four bulked DNA samples based on the four different phenotypic classes (Table 3). Primary screening used a set of 28 microsatellites, distributed across the barley genome (Table 2). Polymorphisms between the bulks containing completely susceptible lines (group 1, Table 3) and those resistant to BaMMV and BaYMV but susceptible to BaYMV-2 (group 2), and those from completely resistant lines (group 3), or resistant to

Table 3 Classification of the mapping population based on the reaction to the different viruses of the barley yellow mosaic virus complex and results of screening with the microsatellite Bmac29 for the detection of genotypes carrying *rym4*

Phenotypic group	BaMMV	BaYMV	BaYMV-2	Number of DH lines	*rym4* (Bmac29)
1	–[a]	–	–	27	172 bp[b]
2	+	+	–	17	158 bp[c]
3	+	+	+	19	158 bp
4	–	+	+	30	172 bp

[a] –, susceptible; +, resistant
[b] 172 bp indicative for the susceptibility encoding allele *Rym4*
[c] 158 bp indicative for the resistance encoding allele *rym4*

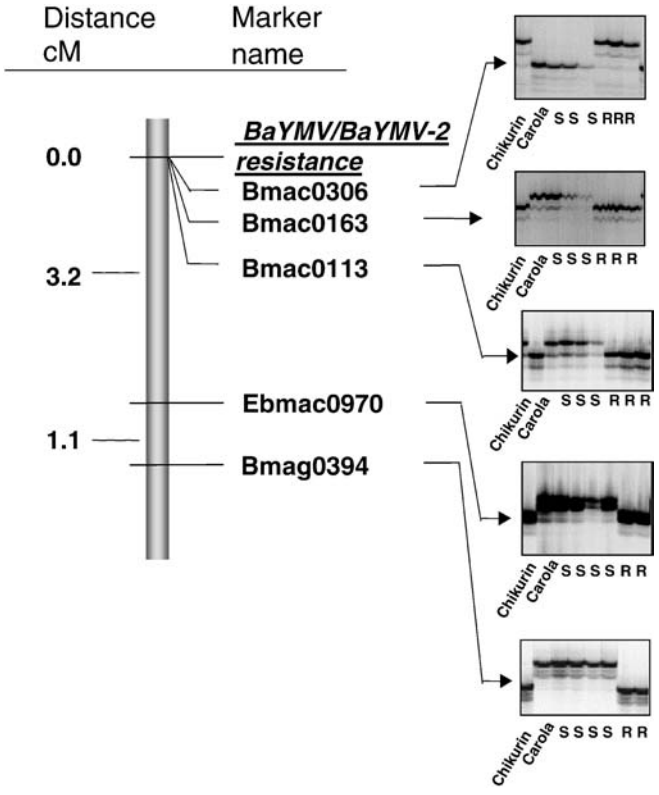

Fig. 3 Partial genetic map of chromosome 5H including BaYMV/BaYMV-2 resistance of Chikurin Ibaraki 1 based on the analysis of 93 DH lines of a complex breeders' cross

BaYMV, and BaYMV-2 (group 4), which should be indicative of linkage to the BaYMV/BaYMV-2 resistance of Chikurin Ibaraki 1, were observed with two SSRs, EBmac0970 and Bmac0113. Both of these are located on chromosome 5H. No informative polymorphic fragments were observed between the bulks with the other 26 SSRs tested. Linkage of the BaYMV/BaYMV-2 resistance of Chikurin Ibaraki 1 to Ebmac0970 and Bmac0113 was confirmed by analysis of the individual lines included in the bulks. Additional SSRs located in this chromosomal region of 5H were then analysed, and well-defined polymorphisms were detected for three additional SSRs – Bmag0394, Bmac0306 and Bmac0163 (Fig. 3). Consequently, linkage analysis on the entire population of 93 DH lines generated the map presented in Fig. 3. It spans 4.3 cM with three markers, Bmac0113, Bmac0306, and Bmac0163, co-segregating with the resistance gene. Our results amply demonstrate the usefulness of SSRs for the identification and mapping of new resistance genes.

Discussion

Given that seven strains of BaYMV and two of BaMMV have been described in Japan (Nomura et al. 1996) and additional variants of BaYMV and BaMMV have been reported in France (Hariri et al. 2000), broadening the genetic base of resistance in modern barley cultivars is an important task for barley breeding. In this respect, intensive screening programmes for resistance have been carried out in Japan (e.g. Kawada and Tsuru 1987; Kawada 1991) and Germany (e.g. Friedt et al. 1985; Ordon et al. 1993). Genetic analyses of different resistances have also been carried out (Takahashi et al. 1973; Götz and Friedt 1993; Ordon et al. 1993; Konishi et al. 1997). However, in these studies different reactions to Japanese soil-borne mosaic-inducing viruses and European isolates of the barley yellow mosaic virus complex were observed (Konishi and Kaiser-Alexnat 2000; Konishi et al. 2002). is described as being susceptible to BaYMV in Japan, while its gamma ray-induced early-heading mutant Ea52 is resistant (Ukai and Yamashita 1980). In Europe, Chikurin Ibaraki 1 is resistant to BaMMV, BaYMV and BaYMV-2, while Ea52 is exclusively resistant against BaYMV and BaYMV-2 but susceptible to BaMMV (Götz and Friedt 1993), suggesting that the pathogenic spectrum of Japanese isolates is different from that in Europe. The same holds true for Ishuku Shirazu, which was used to map *rym3* – originally described in Ea52 – onto the short arm of barley chromosome 5H (Saeki et al. 1999). Similar results on the reaction to European and Japanese strains were obtained for *rym5*, which is completely efficient in Europe (Graner et al. 1999a) but is not effective against strain BaMMV-Na in Japan (Konishi and Kaiser-Alexnat 2000). The resistance locus of Chikurin Ibaraki 1, which is effective against the European BaYMV/BaYMV-2 strains studied here is located in the same chromosomal region as *rym3* from Ishuku Shirazu, which was mapped using Japanese BaYMV (Saeki et al. 1999). Since Chikurin Ibaraki 1 is not resistant in Japan, these results cannot be interpreted conclusively at present. However, it is possible that *rym3* is a complex locus with different specificities to the respective viruses and/or virus strains present in Europe and Japan. Similar results have been observed at the *rym4*/*rym5* locus on chromosome 3HL. While there is allelism with respect to BaMMV (Götz and Friedt 1993), *rym4* confers resistance to BaYMV only, while *rym5* additionally encodes resistance to BaYMV-2 (Graner et al. 1999a, b). It is also possible that the resistance exhibited by carriers of *rym3* (like Ea52, Ishuku Shirazu, or 'Hagane Mugi') to European BaYMV and BaYMV-2 (Saeki et al. 1999) may be based on an additional resistance gene not effective in Japan. In relation to this, Konishi and Furusho (2000) concluded that the resistance of cv. 'Franka' (*rym4*) to Japanese BaYMV is not conferred by *rym4*. Given the interpretation of our results, it is a possibility (hopefully remote) that instead of Chikurin Ibaraki 1, its mutant line Ea52 was erroneously used in our initial cross. To explore this possibility, the parental lines as well as Ea52 were analysed with a set of SSRs. Unfortunately, due to the fact that most of the Chikurin Ibaraki 1 donor genome had been previously bred out of the immediate parents of our DH population and that the SSRs linked to the resistance locus on 5H were, as expected, monomorphic between

Chikurin Ibaraki 1 and its induced mutant Ea52, we remain unable to exclude the above possibility. Nevertheless, although the results raise many questions concerning the relationship between the resistance of Chikurin Ibaraki 1 and *rym3*, as well as the transferability of results obtained in Europe to Japan, they do provide a deeper insight into the basis of resistance in Chikurin Ibaraki 1 to the European members of the barley yellow mosaic virus complex. Thus, we conclude that BaMMV resistance from Chikurin Ibaraki 1 is inherited independently from the BaYMV/BaYMV-2 locus that we have mapped to chromosome 5H. Furthermore, it appears that this BaMMV resistance has been lost during the complex crossings carried out to combine the resistance of Chikurin Ibaraki 1 with superior agronomic traits (Fig. 1). As a result, further investigations are needed to determine whether this additional resistance locus is effective only against BaMMV, as has been shown for *rym9* (Bauer et al. 1997), against BaMMV and BaYMV like *rym4* (Graner et al. 1999b) or against BaMMV, BaYMV and BaYMV-2 like *rym5* (Graner et al. 1999b). Two resistance genes against BaYMV, i.e. *rym1* and *rym5*, have been detected in some germplasm as well e.g. Mokusekko 3 (Konishi et al. 1997).

Aside from the genetic basis of resistance against barley yellow mosaic viruses, our results clearly demonstrate the usefulness of SSRs in gene mapping, a conclusion which has already been drawn in many species (Yu et al. 1994; Blair and McCouch 1997; Mudge et al. 1997; Peng et al. 1999; Xu et al. 1999; Iqbal et al. 2001; Scheurer et al. 2001). In the present study, Bmac29 facilitated the identification of progenies carrying a known resistance gene (*rym4*) (Graner et al. 1999a, Fig. 2) and this was a prerequisite for mapping the Chikurin Ibaraki 1 resistance. In comparison to restriction fragment length polymorphisms (RFLPs) (Graner et al. 1991), SSRs facilitate faster and easier mapping, and in contrast to AFLPs (Vos et al. 1995), they can be directly used in plant breeding without the need for further development into sequence-tagged sites (Powell et al. 1996). However, it is pertinent to note that the available mapped barley SSRs tend to cluster towards the centromeric region of the genetic map (Ramsay et al. 2000) (not the physical map, G. Künzel et al. personal communication.) and as a result, differences in genetic distance and rearrangements can sometimes be observed (Ramsay et al. 2000; Macaulay et al. 2001). In the case of the DH population analysed here, the genetic distance between the linked SSRs comprises only 4.3 cM, compared to the 44 cM in Ramsay et al. (2000), which suggests that recombination in this region is suppressed in our DH population.

Due to their close linkage, these SSRs will prove to be useful tools for marker-based selection for the BaYMV/BaYMV-2 resistance of Chikurin Ibaraki 1 and will assist in combining this locus with additional resistance genes against the barley yellow mosaic virus complex for which easy-to-handle PCR-based markers have already been established (Bauer et al. 1997; Schiemann et al. 1997; Graner et al. 1999a; Werner et al. 2000a). Indeed, by developing this breeders DH population, pyramiding of resistance genes has already been carried out. As *rym4* was crossed into the population immediately prior to the recovery of the DHs 25% of these lines are expected to carry both *rym4* and the resistance from Chikurin Ibaraki 1 in the homozygous recessive Chikurin Ibaraki 1 condition, i.e. they should be resistant against BaMMV, BaYMV and BaYMV-2. As can be seen in Table 3 this expectation was observed ($\chi^2 = 1.036$, $0.20 < P < 0.50$). Attempts to pyramid different resistance genes against the barley yellow mosaic virus complex are currently being carried out (Saeki et al. 1999; Pellio et al. 2000; Werner et al. 2000b). In other crops such as rice (Huang et al. 1997; Hittalmani et al. 2000; Singh et al. 2001), wheat (Dweikat et al. 1997; Kloppers and Pretorius 1997; Liu et al. 2000) and pepper (Caranta et al. 1996), attempts to pyramid resistance genes are also being pursued with the objective of developing more robust genetic resistance.

With respect to European barley breeding, the closely linked SSR markers described here, which exhibit DI values of 0.49–0.66 (Ramsay et al. 2000), represent ideal tools for the rapid marker-assisted introgression (Ordon et al. 1999) of Chikurin Ibaraki 1 resistance followed by marker-based selection in barley breeding programmes. This exercise is already being carried out extensively for *rym5* using Bmac29 (Schiemann and Backes 2000). In comparison to Chikurin Ibaraki 1, the breeders population used here for mapping is already much better adapted and higher yielding than the donor. As a result, it is realistic to expect that cultivars carrying the BaYMV/BaYMV-2 resistance of Chikurin Ibaraki 1 will be released quickly into the community, thereby broadening the genetic base of resistance against BaYMV-2, which is currently exclusively based on *rym5* (Friedt et al. 2000).

Acknowledgements We acknowledge the excellent technical assistance of Natalia Lest, Franziska Müller, and Roland Kürschner. We thank Dr. Reinhard Hemker, Nickerson Pflanzenzucht, Dr. Manfred Herrmann and Dr. Jens Vaupel, Deutsche Saatveredelung, and Adalbert Bund, Saatzucht Oberlimpurg (all Germany) for carrying out field tests for virus resistance. Furthermore, thanks are due to Dr. Frank Rabenstein, BAZ, Aschersleben for providing ELISA antisera. This study was supported by the Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft (BMV-EL) and the Gemeinschaft zur Förderung der privaten deutschen Pflanzenzüchtung e.V., Bonn (GFP, G82/98 HS).

References

Anonymous (2001) Beschreibende Sortenliste für Getreide, Mais, Ölfrüchte, Leguminosen (grosskörnig), Hackfrüchte (ausser Kartoffeln). Bundessortenamt (Hrsg). Landbuch Verlagsgesellschaft, Hannover, Germany

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

Blair MW, McCouch SR (1997) Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene *xa-5*. Theor Appl Genet 95:174–184

Caranta C, Palloix A, Gebre-Selassie K, Lefebvre V, Moury B, Daubèze AM (1996) A complementation of two genes originating from susceptible *Capsicum annuum* lines confers a new and complete resistance to pepper veinal mottle virus. Phytopathology 86:739–743

Doyle JF, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 121:13–15

Dweikat I, Ohm H, Patterson F, Cambron S (1997) Identification of RAPD markers for 11 Hessian fly resistance genes in wheat. Theor Appl Genet 94:419–423

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

Friedt W, Huth W, Mielke H, Züchner S (1985) Resistenzträger gegen Barley Yellow Mosaic Virus. Nachrichtenbl Dtsch Pflanzenschutzdienst 37:129–135

Friedt W, Werner K, Ordon F (2000) Genetic progress as reflected in highly successful and productive modern barley cultivars. In: Logue S (ed) Proc 8th Int Barley Genet Symp, vol I. Department of Science, Adelaide, Australia, pp 271–279

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, Bauer E (1993) RFLP mapping of the *ym4* virus resistance gene in barley. Theor Appl Genet 86:689–693

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

Graner A, Bauer E, Kellermann A, Proeseler G, Wenzel G, Ordon F (1995) RFLP analysis of resistance to the barley yellow mosaic virus complex. Agronomie 15:475–479

Graner A, Bauer E, Chojecki J, Tekauz A, Kellermann A, Proeseler G, Michel M, Valkov V, Wenzel G, Ordon F (1996) Molecular mapping of disease resistance in barley. In: Scoles G, Rossnagel B (eds) Proc 5th Int Oat Conf 7th Int Barley Genet Symp, vol I. University Extension Press, Saskatoon, Sask., pp 253–255

Graner A, Streng S, Kellermann A, Schiemann A, Bauer E, Waugh R, Pellio B, Ordon F (1999a) Molecular mapping of the *rym5* locus encoding resistance to different strains of the Barley Yellow Mosaic Virus Complex. Theor Appl Genet 98:285–290

Graner A, Streng S, Kellermann A, Proeseler G, Schiemann A, Peterka H, Ordon F (1999b) Molecular mapping of genes conferring resistance to soil-borne viruses in barley. An approach to promote understanding of host-pathogen interactions. J Plant Dis Protect 106:405–410

Graner A, Michalek W, Streng S (2000) Molecular mapping of genes conferring resistance to viral and fungal pathogens. In: Proc 8th Int Barley Genet Symp, vol 1. Adelaide, Australia, pp 45–53

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

Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100:1121–1128

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 95:313–320

Huth W (1989) Ein weiterer Stamm des Barley Yellow Mosaic Virus in der Bundesrepublik Deutschland. Nachrichtenbl Dtsch Pflanzenschutzdienst 40:49–55

Huth W (1991) Verbreitung der Gelbmosaikviren, BaYMV, BaMMV und BaYMV-2 und Screening von Gerstensorten auf Resistanz gegenüber BaYMV-2. Nachrichtenbl Dtsch Pflanzenschutzdienst 43:233–237

Huth W, Adams MJ (1990) Barley Yellow Mosaic Virus (BaYMV) and BaYMV-M: two different viruses. Intervirology 31:38–42

Huth W, Lesemann DL (1978) Eine für die Bundesrepublik Deutschland neue Virose der Wintergerste. Nachrichtenbl Dtsch Pflanzenschutzdienst 36:184–185

Iida Y, Konishi T (1994) Linkage analysis of a resistance gene to barley yellow mosaic virus strain II in two rowed barley. Breed Sci 44:191–194

Iida Y, Ban T, Konishi T (1999) Linkage analysis of the rym6 resistance gene to Japanese strain II of barley yellow mosaic virus (BaYMV-II) in barley. Barley Genet Newsl 29:31–32

Iqbal MJ, Meksem K, Njiti VN, Kassem A, Lightfoot DA (2001) Microsatellite markers identify three additional quantitative trait loci for resistance to soybean sudden-death syndrome (SDS) in Essex × Forrest RILs. Theor Appl Genet 102:187–192

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

Kawada N, Tsuru M (1987) Genetics and breeding of resistance to barley yellow mosaic virus. In: Proc 5th Int Barley Genet Symp. Barley Genet 5:651–657

Kloppers FJ, Pretorius ZA (1997) Effects of combinations amongst genes *Lr13*, *Lr34* and *Lr37* on components of resistance in wheat to leaf rust. Plant Pathol 46:737–750

Konishi T, Furusho M (2000) German barley cv. Franka posseses two different resistance genes to barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV). Barley Genet Newsl 30:55–58

Konishi T, Kaiser-Alexnat R (2000) Reaction of barley accessions to BaYMV and BaMMV in Japan, compared with data in Germany. Barley Genet Newsl 30:58–62

Konishi T, Ban T, 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

Konishi T, Ordon F, Furusho M (2002) Reactions of barley accessions carrying different rym genes to BaYMV and BaMMV in Japan and Germany. Barley Genet Newsl 32:46–48

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 1:174–181

Liu J, Liu D, Tao W, Li W, Wang Chen P, Cheng S, Gao D (2000) Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. Plant Breed 119:21–24

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

Macaulay M, Ramsay L, Powell W, Waugh R (2001) A representative, highly informative genotyping set of barley SSRs. Theor Appl Genet 102:801–809

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

Mudge J, Cregan PB, Kenworthy JP, Kenworthy WJ, Orf JH, Young ND (1997) Two microsatellite markers that flank the major soybean cyst nematode resistance locus. Crop Sci 37:1611–1615

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 144:103–107

Ordon F, Friedt W (1993) Mode of inheritance and genetic diversity of BaMMV-resistance of exotic barley germplasms carrying genes different from '*rym4*'. Theor Appl Genet 86:229–233

Ordon F, Friedt W (1994) Agronomic traits of exotic barley germplasms resistant to soil-borne mosaic-inducing viruses. Genetic Resources Crop Evol 41:43–46

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, Bauer E, Friedt W, Graner A (1995) Marker-assisted selection for the *ym4* BaMMV resistance gene in barley using RAPDs. Agronomie 15:481–485

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

Ordon F, Schiemann A, Pellio B, Dauck V, Bauer E, Streng S, Friedt W, Graner A (1999) Application of molecular markers in breeding for resistance to the barley yellow mosaic virus complex. J Plant Dis Protect 106:256–264

Pellio B, Werner K, Friedt W, Graner A, Ordon F (2000) Resistance to the barley yellow mosaic virus complex – from Mendelian genetics towards map based cloning. Czech J Genet Plant Breed 36:84–87

Peng JH, Fahima T, Röder MS, Li YC, Dahan A, Grama A, Ronin YI, Kororl AB, Nevo E (1999) Microsatellite tagging of the stripe-rust resistance gene *YrH52* derived from wild emmer wheat, *Triticum dicoccoides*, and suggestive negative crossover interference on chromosome 1B. Theor Appl Genet 98:862–872

Powell W, Barid E, Booth A, Lawrence M, McAulay M, Bonar N, Young G, Thomas WTB, McNicol JW, Waugh R (1996) Single locus and multi-locus molecular assays for barley breeding research. In: Scoles G, Rossnagel B (eds) Proc 5th Int Oat Conf 7th Int Barley Genetics Symp. University Extension Press, Saskatoon, Sask., pp 174–181

Proeseler G, Hartleb H, Kophanke D, Lehmann CO (1989) Resistenzeigenschaften im Gersten- und Weizensortiment Gatersleben. 27. Prüfung von Gersten auf ihr Verhalten gegenüber 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

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

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

Scheurer KS, Friedt W, Huth W, Waugh R, Ordon F (2001) QTL analysis of tolerance to a German strain of BYDV-PAV in barley (*Hordeum vulgare* L.). Theor Appl Genet 103:1074–1083

Schiemann A, Backes G (2000) The use of molecular markers in practical barley breeding. In: Proc 8th Int Barley Genet Symp, vol 3. Adelaide, Australia, pp 42–44

Schiemann A, Graner A, Friedt W, Ordon F (1997) Specificity enhancement of a RAPD marker linked to the BaMMV/BaYMV resistance gene *ym4* by randomly added bases. Barley Genet Newsl 27:63–65

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS (2001) Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. Theor Appl Genet 102:1011–1015

Takahashi R, Hayashi J, Inouye T, Moriya I, Hirao C (1973) Studies on resistance to yellow mosaic disease in barley. I. Tests for varietal reactions and genetic analysis of resistance to the disease. Ber Ohara Inst 16:1–17

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

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

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

Vos P, Hogers R, Bleeker M, Reijans M, Van de Le T, Hornes M, Frijiters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

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

Werner K, Friedt W, Ordon F (2000b) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex based on molecular markers and DH-lines. In: Proc 8th Int Barley Genet Symp, vol 2. Adelaide, Australia, pp 200–202

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

Xu ML, Melchinger AE, Xia XC, Lübberstedt T (1999) High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR, and AFLP markers. Mol Gen Genet 261:574–581

Yu YG, Saghai Maroof MA, Buss GR, Maughaun PJ, Tolin SA (1994) RFLP and microsatellite mapping of a gene for soybean mosaic virus resistance. Phytopathology 84:60–64