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Effects of barley yellow mosaic disease resistant gene *rym1* on the infection by strains of *Barley yellow mosaic virus* and *Barley mild mosaic virus*

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Abstract Although a Chinese landrace of barley, Mokusekko 3, is completely resistant to all strains of *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV), and is known to have at least two resistant genes, *rym1* and *rym5*, only *rym5* has been utilized for BaYMV resistant barley breeding in Japan. In order to clarify the effect of *rym1* on BaYMV and BaMMV, and to utilize the gene for resistant barley breeding, the susceptibilities of only *rym1* carrying breeding lines against BaYMV and BaMMV were investigated. In the assessment of resistance to BaYMV-I, 341 F_2 populations derived from a cross between the resistant line Y4 with only *rym1* and the susceptible cv Haruna Nijo shows that the segregation loosely fits a 1R:3S ratio ($0.05 > P > 0.01$), suggesting that the resistance is controlled by a single recessive gene, *rym1*. Further, none of the F_3 lines derived from the nine resistant F_2 plants showed any disease symptoms in the field infected by BaYMV-I. The same nine F_3 lines showed almost the same agronomic characters in the field infected by BaYMV-III as those in the uninfected field, apart from the symptom of showing numerous mosaics. This result indicates that the gene *rym1* has an acceptable level of resistance to BaYMV-III. In the assessment of resistance to BaYMV-II, BaMMV-Ka1 and -Na1, an artificial infection method was adopted and the susceptibilities to those viruses were investigated. Although the control varieties, Ko A and Haruna Nijo, were infected with all of them, the *rym1* gene carrying BC_2F_3 lines were completely resistant to all strains. In summary, *rym1* is completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1, and has an acceptable level of resistance to BaYMV-III. This study concludes with a discussion of the reason why the important resistance gene *rym1* was eliminated along with resistant cultivars during breeding for resistance to BaYMV.

Keywords *Barley yellow mosaic virus* · *Barley mild mosaic virus* · Strain · Resistance gene · *rym1* · *rym5*

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Introduction

East Asian and European winter barley cultivars are seriously damaged by BaYMV and BaMMV (Iketa and Kawai 1940; Huth and Lesemann 1978). The viruses are transmitted by a soil-borne fungus, *Polymyxa graminis* (Toyama and Kusaba 1970), and barley seedlings show extreme symptoms such as yellow streaking and brown necrotic patches. Based on the serological properties (Huth et al. 1984; Kashiwazaki et al. 1989) and the nucleotide sequences of the capsid proteins (Kashiwazaki et al. 1992; Schlichter et al. 1993), BaYMV and BaMMV have been classified into different subgroups within the bymovirus group. In Japan, BaYMV occurrence is widespread and the virus causes serious damage to two-rowed malting barley (Kashiwazaki et al. 1990). Based on pathogenicity tests on different barley cultivars, BaYMVs identified in Japan are generally classified into three strains, BaYMV-I, -II and -III (Kashiwazaki et al. 1989), while in Europe, they are classified into two strains, BaYMV-1 and -2 (Huth 1989). In Japan, two strains of BaMMV, -Ka1 and -Na1, have been isolated (Nomura et al. 1996).

The most common approach for the prevention of infection with BaYMV and BaMMV is the introgression of the resistance genes identified in barley germplasm accessions into modern barley cultivars. A Chinese six-rowed barley landrace, Mokusekko 3, is unique in being completely resistant to all the strains of BaYMV and BaMMV in Japan (Kashiwazaki et al. 1989; Iida et al. 1992; Nomura et al. 1996). Konishi et al. (1997) have indicated that at least two resistance genes, *rym1* and

rym5, confer resistance to BaYMV in Mokusekko 3. In Japan, using Mokusekko 3 as a cross parent, many BaYMV-resistant malting barley cultivars have been developed. Though Mokusekko 3 harbors at least two BaYMV resistance genes, only *rym5* was used for barley breeding against BaYMV. However, the breakdown of its resistance was detected in the field by the appearance of a new virus strain, BaYMV-III (Ogawa et al. 1987). Another gene resistant to all strains of BaYMV is *rym3*. The *rym3* gene was found in Haganemugi and Ea 52 (Ukai and Yamashita 1980; Kawada 1991) and displays resistance to all strains of BaYMV in Japan. However, *rym3* is not effective against BaMMV. In consequence, the effects of the *rym5* and *rym3* gene on infection by strains of BaYMV and BaMMV in Japan have been reported, but the effect of the *rym1* gene is unknown. In addition, an RFLP marker linked to *rym5* (Graner et al. 1995; 1999) and *rym3* (Saeki et al. 1999) has already been reported. RFLP linkage maps have recently been reported for the location of the *rym1* locus and *rym5* locus in Mokusekko 3 (Miyazaki et al. 2001). In addition, a new QTL for BaYMV resistance was identified derived from Mokusekko 3 (Miyazaki et al. 2001). Thus, this present study first raises the issue of only *rym1* was introgressed into modern malting barley cultivars using DNA markers, and investigates the effect of the *rym1* gene on infection by strains of BaYMV and BaMMV. This is followed by a discussion of the reason why the important resistance gene *rym1* was eliminated, along with resistant cultivars, during breeding for resistance to BaYMV.

Materials and methods

DNA extraction and screening by Southern hybridization

DNA was extracted from the mature leaves of the plants and their parents, as described in the standard protocol of the CTAB method (Murray and Thompson 1980). Then, 3 μg of each DNA sample was individually digested with *Bam*HI or *Dra*I. These digested

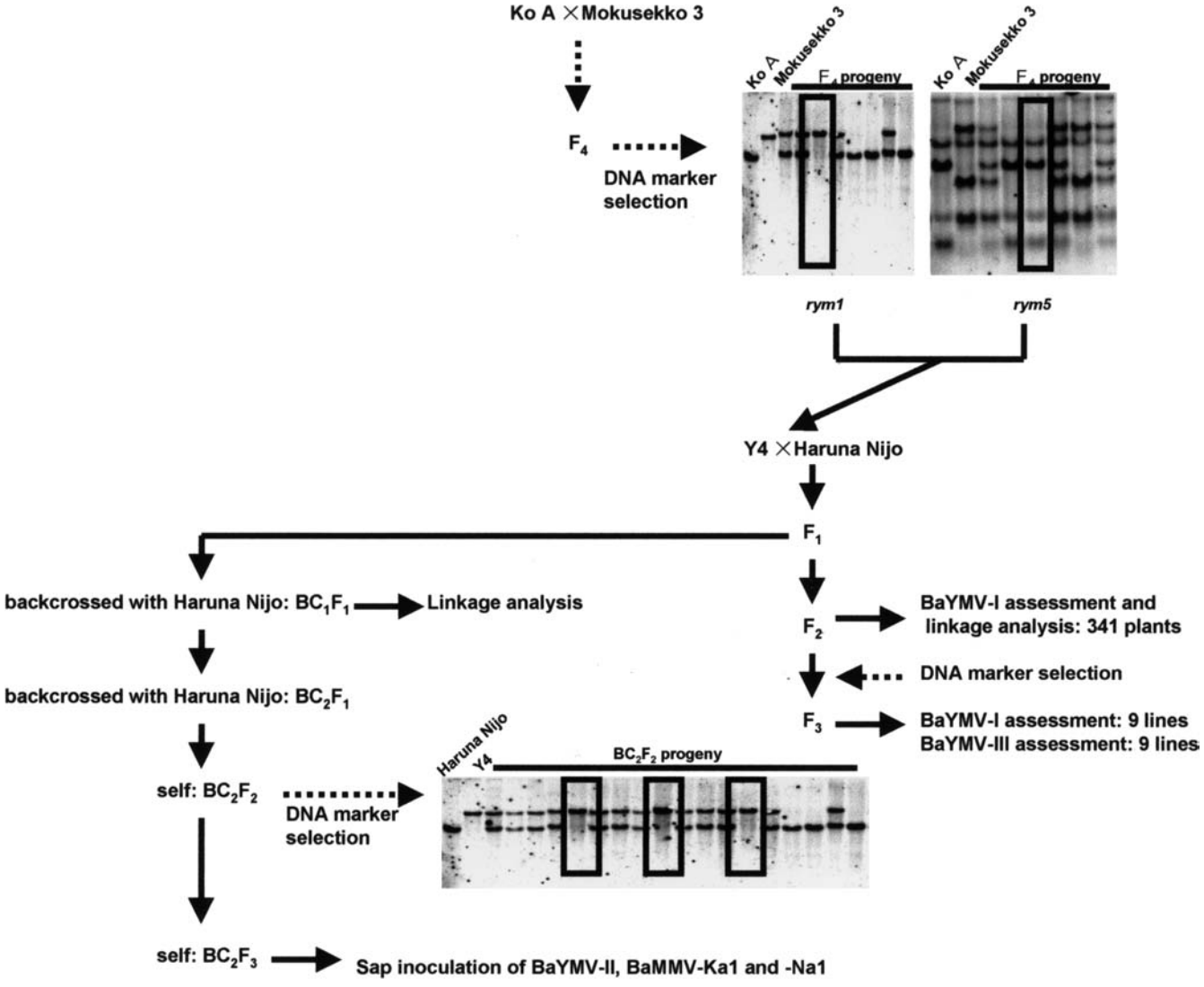


Fig. 1 Selection of progeny carrying a BaYMV resistance *rym1* gene from Mokusekko 3 by DNA markers, and cross combinations for the assessment to strains of BaYMV and BaMMV

Table 1 Interrelationship between the reaction to BaYMV-I and DNA marker genotypes in F_2 progeny of Y4 (R, aa) × Haruna Nijo (S, AA). Chi-square test: χ^2 (1R:3S) = 5.21, χ^2 (MWG2134 for 1:2:1) = 2.34

MWG2134[a]	Reaction of BaYMV-I[b]		
	R	S	Total
AA	1	94	95
Aa	1	170	171
aa	65	10	75
Total	67	274	341

[a] AA, Haruna Nijo type; Aa, Hetero type; aa, Mokusekko 3 type
[b] R, resistant; S, susceptible

DNAs were electrophoresed on a 0.8% agarose gel and transferred to nylon membranes by the capillary method (Sambrook et al. 1989). The prehybridization, hybridization, detection and probe labeling procedures were performed according to the Gene Images system (Amersham Pharmacia Biotech) manual.

Plant materials and resistance assessment against BaYMV-I and -III

An RFLP linkage map and significant QTLs for *rym1* and *rym5* were reported by Miyazaki et al. (2001). Only plant materials of the *rym1* donor were used, derived from F_4 lines of the same population for QTL mapping of BaYMV resistance by Miyazaki et al. (2001). A BaYMV-resistant F_4 progeny derived from Ko A×Mokusekko 3 carrying only *rym1* was screened by DNA markers linked to *rym1* (MWG2134, MWG2159 and MWG58) and linked to *rym5* (ABC172) as illustrated in Fig. 1. Its progeny, Y4, carrying only *rym1* isolated from Mokusekko 3, proved to be homozygous for resistance. A total of 341 F_2 populations were derived from a cross between the resistant line Y4 and the susceptible cv Haruna Nijo. These were grown in a field infected with only BaYMV-I, and segregation of the reaction was investigated in the 1999–2000 season. Furthermore, nine resistant F_2-derived F_3 lines (40 seedlings/line) were grown in the same field and the reaction was investigated in the 2000–2001 season. Parents of the cross, susceptible checks and differential genotypes were planted at the same time. All of the assessment plants were individually harvested and their agronomic characters were examined. The disease reaction was evaluated individually based on the mosaic symptoms on the leaves. If at least one leaf with mosaic symptoms was detected, the plant was scored as susceptible.

In the assessment of resistance to BaYMV-III, the same nine F_3 lines were derived from F_2 plants that are resistant to BaYMV-I and selected homozygotes, using DNA markers linked to *rym1* (Fig. 1). In the 2000–2001 season, 40 seedlings per F_3 line were grown in a field infected with only BaYMV-III and investigated for their reaction to BaYMV-III. The assessment of resistance to BaYMV-III was based on the mosaic symptoms and yellowing on the leaves. The scoring of symptoms and assessment used the observed values of 0.0 to 6.0. All assessment plants were individually harvested and their agronomic characters were examined.

Plant materials and sap inoculation of BaYMV-II, BaMMV-Ka1 and -Na1

The infection rate of sap inoculation is lower than field infection. In addition, the infection rate of sap inoculation is much more effective to genotypes (Kashiwazaki et al. 1989). On the other hand, we have a variety of information of sap inoculation on Haruna Nijo. Therefore, plant materials were screened by DNA markers linked to *rym1*, derived from Y4 × Haruna Nijo, and two times backcrossed with Haruna Nijo and the selfed BC_2F_3 lines (Fig. 1). Fifteen BC_2F_2 lines were selected by DNA markers, and these seeds were bulked and used for plant materials. Inocula were obtained by grinding infected leaves in 0.1 M phosphate buffer (pH 7.0) containing 1 mM of potassium cyanide and carborundum (Kashiwazaki et al. 1989). The leaf surfaces of barley seedlings were grown in growth chambers controlled at 13–15 °C with natural lighting. The symptoms of the plants were observed during the

Table 2 Agronomic characters of susceptible cultivars and the *rym1* gene-carrying lines grown in an infested field by BaYMV-I or -III

Item	Variety/line	Culm length (cm)	Panicle length (cm)	Number of grains per panicle	Panicle number
Grown in an uninfested field	Haruna Nijo,	99.8±4.5	5.9±0.4	25.9±1.7	12.2±3.1
	Mikamo Golden	99.0±5.0	6.3±0.3	26.7±1.7	11.0±2.6
Grown in an infested field by BaYMV-I	Haruna Nijo	63.5±4.1	5.1±0.3	20.7±1.8	6.3±2.2
	SA22	88.0±4.3**	5.1±0.3	20.4±2.0	8.3±1.8*
	SA52	99.0±5.8**	6.2±1.0**	23.8±4.3*	10.6±3.7**
	SA58	97.2±6.0**	6.0±0.5**	23.0±2.4*	11.5±2.1**
	SA75	99.3±4.5**	6.2±0.4**	22.8±2.7*	10.7±1.6**
	SA141	82.5±3.1**	5.1±0.3	20.4±1.3	8.1±2.0*
	SA144	101.9±3.8**	6.3±0.5**	25.1±2.4**	10.2±3.3**
	SA154	94.2±4.4**	6.0±0.8**	22.7±2.9	10.0±2.7**
	SA184	89.0±7.1**	5.5±0.5	21.1±2.6	7.5±3.0
	SA201	85.1±6.6**	5.3±0.6	20.8±3.3	8.8±2.6*
Grown in an infested field by BaYMV-III	Mikamo Golden	79.3±3.4	5.6±0.2	24.3±0.5	7.3±1.7
	SA22	93.8±3.8**	5.8±0.3	21.5±1.6	9.0±1.9*
	SA52	93.8±7.4**	6.0±0.9	25.1±4.5	9.3±3.1*
	SA58	96.2±6.4**	5.8±0.6	23.8±3.9	9.3±2.8*
	SA75	94.2±5.8**	6.1±0.5*	25.3±2.7	11.2±4.3**
	SA141	83.3±6.6	5.0±0.6	22.3±3.1	9.4±3.7*
	SA144	95.3±7.7**	6.3±0.5**	28.2±2.5**	10.7±3.8**
	SA154	83.9±8.1	5.3±0.6	24.9±3.4	9.6±2.4*
	SA184	85.9±12.9	5.5±0.9	22.4±4.2	8.9±1.9*
	SA201	90.1±7.7*	5.3±0.7	22.7±3.7	8.0±3.6

** = significant at the 0.01 and * = significant at the 0.05 probability level

2 months after inoculation. The disease reaction was evaluated in each individual plant, as a substrate according to the method of Kashiwazaki et al. (1989). At least 20 seedlings of each line were inoculated in one experiment, and all lines were tested twice at 1-month intervals in the winter.

Linkage analysis of *rym1* on chromosome 4H

A 341 F_2 population of Y4 × Haruna Nijo, with DNA markers linked to *rym1* (MWG2134, MWG2159 and MWG58) and to *rym5* (ABC172), were individually examined and grown in a field infected with BaYMV-I, and segregation of the reaction was investigated. A resistance gene *rym1* of Mokusekko 3 is loosely linked to the morphological marker *K* for a hooded lemma (Takahashi et al. 1973). Further analysis revealed that the *rym1* locus was located on chromosome 4H and linked to the morphological markers *K* and *glf3* (glossy leaf 3) (Konishi et al. 1997). We investigated the segregation of the blue aleurone (*Bl*); however, it was difficult to distinguish the homozygous white aleurone from the homozygous light blue in F_2 progenies. On the other hand, heterozygous ones were easily distinguishable from the white aleurone. Consequently, the segregation of the morphological marker *Bl* was performed using 95 BC_1F_1 populations of Y4 × Haruna Nijo (Fig. 1), since the aleurone of the parent Y4 was slightly bluish.

Results

Effect of *rym1* resistance to BaYMV-I

Segregation of the reaction to BaYMV-I in F_2 progeny of Y4 × Haruna Nijo was examined. Those progeny segregating susceptible plants were treated as susceptible (S) as opposed to resistant homozygous ones (R) in the test for segregation. Table 1 shows that the segregation ratio was 67R:274S ($\chi^2 = 5.21$, $P = 0.02$); there is an excess of susceptible plants and the chi-square value was above the normal threshold of 3.84 for a 1R:3S segregation corresponding to $P = 0.05$. We consider that there are two reasons for this: (1) to consider these resistances to be governed by both the *rym1* gene and a partially dominant one; therefore the segregation did not fit a 1R:3S ratio in the F_2 population, (2) to consider the possibility to distinguish the physiological stunt by wet injury/frost injury to be susceptible; therefore there is an excess of susceptible plants. Further, the result of the nine F_3 lines (40 seedlings/line) derived from resistant F_2 plants have been

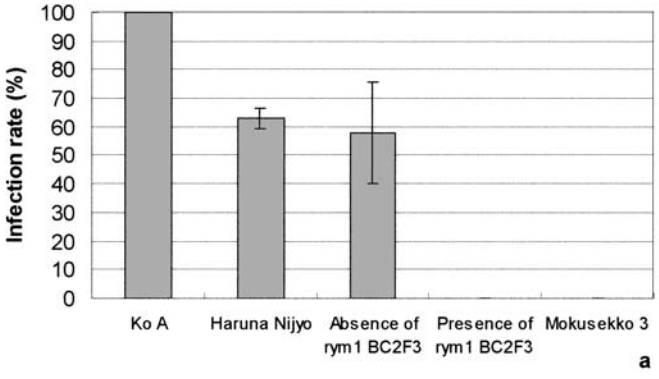
Fig. 2a, b Effect of the *rym1* gene's resistance to BaYMV-I. **a** These were grown in a field infected with only BaYMV-I: left, carrying a *rym1* line; right, susceptible control Haruna Nijo. **b** These were grown in an infested field and harvested. There were significant differences between the *rym1* gene-carrying lines and the susceptible Haruna Nijo grown in a field infested by BaYMV-I. The susceptible cultivar Haruna Nijo showing dwarfed: left, carrying a *rym1* line grown in an infested field; center, susceptible control Haruna. Nijo grown in an infested field; right, susceptible control Haruna Nijo grown in an uninfested field

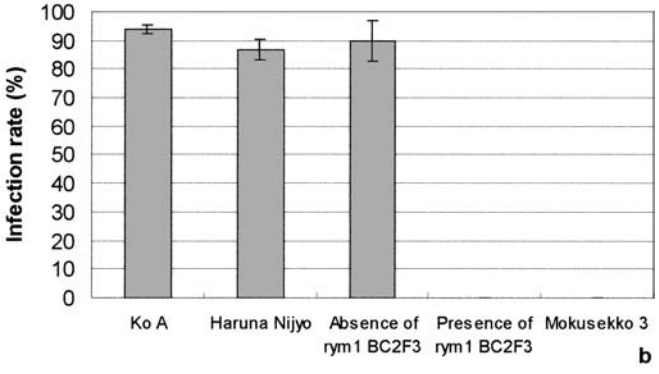
Fig. 3a–d Effect of the *rym1* gene's resistance to BaYMV-III. **a** The infection of barley seedlings grown in the field by BaYMV-III was so effective that the resistant plants were easily distinguished from the susceptible ones. **b** The assessment of resistance to BaYMV-III, all lines were susceptible, with the symptom showing numerous mosaics. **c** The assessment of resistance to BaYMV-III; there was very mild yellowing, which was similar to the resistant cultivar: left, susceptible control Mikamo Golden; right, carrying a *rym1* line. **d** These were grown in an infested field and harvested. There were significant differences between the *rym1* gene-carrying lines and the susceptible Mikamo Golden grown in a field infested by BaYMV-III. The susceptible cultivar Mikamo Golden is shown dwarfed: left, carrying a *rym1* line grown in an infested field; center, susceptible control Mikamo Golden-grown infested field; right, susceptible control Mikamo Golden-grown in an uninfested field

Table 3 Reaction of cultivars and lines to BaYMV-III

Cultivars and Lines	Resistant gene	Reaction to BaYMV-I[a]	Score of mosaic	Score of yellowing
Amagi Nijo	–	S	6.0	5.5
Haruna Nijo	–	S	6.0	4.0
Mikamo Golden	*rym5*	R	5.0	4.0
Mokusekko 3	*rym1, rym5*	R	0.0	0.0
Kanto Nijo 34	*rym3, rym5*	R	0.0	1.0
Kanto Nijo 29	*rym3*	R	0.0	0.5
SA 22	*rym1*	R	5.0	2.0
SA 52	*rym1*	R	4.5	2.5
SA 58	*rym1*	R	4.5	1.5
SA 75	*rym1*	R	4.5	1.0
SA 141	*rym1*	R	4.5	1.5
SA 144	*rym1*	R	4.5	1.5
SA 154	*rym1*	R	4.5	2.0
SA 184	*rym1*	R	4.5	2.0
SA 201	*rym1*	R	4.5	1.5

[a] R, resistant; S, susceptible





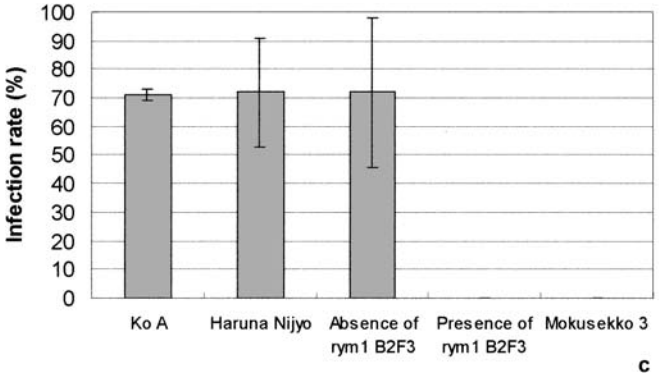


Fig. 4a–c Effect of the *rym1* gene's resistance to BaYMV-II, BaMMV-Ka1 and -Na1. Against the assessment of resistance to BaYMV-II, BaMMV-Ka1 and -Na1, all strains of the susceptible controls Ko A and Haruna Nijo were entirely infected. However, the *rym1* gene-carrying lines did not show any disease symptoms. **a** infection rate of BaYMV-II; **b** infection rate of BaMMV-Ka1; **c** infection rate of BaMMV-Na1

re-tested and none of the F_3 lines showed any disease symptoms and the resistance did not segregated in the F_3 lines. Consequently, it is reasonable to consider this resistance to be controlled by a multiple genes rather than a single recessive gene. All assessment plants were individually harvested and their agronomic characters, i.e. culm length, panicle number, panicle length and number of grains per panicle, were examined. There were significant differences between the *rym1* gene carrying resistant lines and the susceptible Haruna Nijo, in the characteristics tested (Table 2). The culm lengths were especially highly significant, the susceptible Haruna Nijo being dwarfed (Fig. 2).

Effect of *rym1* resistance to BaYMV-III

The infection of barley seedlings grown in the field by BaYMV-III was so effective that the resistant lines were easily distinguishable from the susceptible ones (Fig. 3a). The seedlings of the susceptible controls were entirely infected. The susceptible control Amagi Nijo, Haruna Nijo displayed severe mosaicism (a symptom score of 6.0), and severe yellowing (yellowing leaf scores of 5.5 and 4.0, respectively). The other susceptible control, Mikamo Golden, also displayed mosaicism (a symptom score of 5.0) and yellowing (a yellowing leaf score of 4.0). This indicates that Mikamo Golden was introgressed into only the *rym5* gene from Mokusekko 3 in it's breeding process. All of the F_3 lines were susceptible, with symptom scores of 4.5 to 5.0, showing numerous mosaics which were similar to those on the susceptible cultivar (Table 3 and Fig. 3b). However, the yellowing of leaf scores of 1.0 to 2.0, indicated mild yellowing, which was similar to the resistant cultivars (Table 3 and Fig. 3c). All assessment plants were individually harvested and their agronomic characters, i.e. culm length, panicle number, panicle length and number of grains per panicle, were examined. There were significant differences between the *rym1* gene-carrying lines and the susceptible Mikamo Golden in the characteristics tested (Table 2). In particular, the culm lengths were sig-

Table 4 Linkage data for the BaYMV resistance gene *rym1* and markers on chromosome 4H in F_2 and BC_1F_1 progeny of Y4 × Haruna Nijo

Test No.[a]	Generation	Gene pair		Segregation	χ^2L	Recombination value (%)
		A	B			
1	F_2	MWG2134	*rym1*	94:1/170:1/10:65	215.5	4.17±1.1
2	F_2	MWG2159	*rym1*	94:1/168:3/12:63	199.1	5.47±1.3
3	F_2	MWG058	*rym1*	97:1/166:5/11:61	189.6	5.86±1.3
4	F_2	ABC172	*rym1*	59:8/156:40/59:19	2.13	43.3±3.3 (Independent)
5	BC_1F_1	MWG2134	*Bl*	87:8	–	8.42

[a] Test nos. 1–4: AABb:AAbb/AaBb:Aabb/aaBb:aabb. AA: Haruna Nijo type, Aa: Hetero type, aa: Mokusekko 3 type; Bb: susceptible, bb: resistant. Test no. 5: AaBb : Aabb. AA: Haruna Nijo type, Aa: Hetero type; Bb: blue aleurone, bb: white aleurone

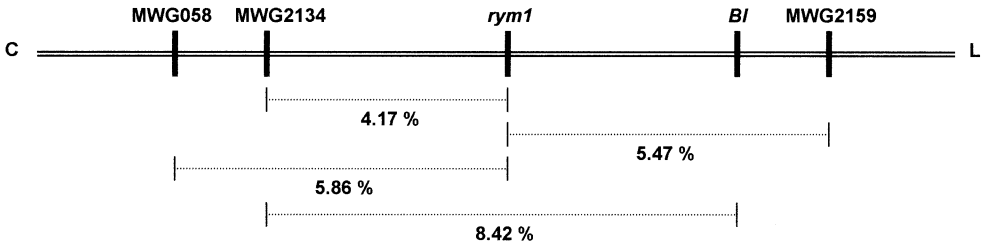


Fig. 5 A linkage map of chromosome 4H containing *rym1* for all strains of BaYMV and BaMMV resistance

nificantly different, the susceptible Mikamo Golden being dwarfed (Fig. 3d).

Effect of *rym1* resistance to BaYMV-II, BaMMV-Ka1 and -Na1 infection

The infected plants developed systemic symptoms such as faint mosaicism, mild mosaicism, mosaicism, necrosis, yellowing and severe mosaicism in sap inoculation tests of BaYMV-II, BaMMV-Ka1 and -Na1. As for the reaction to BaYMV-II, the susceptible control Ko A displayed severe mosaicism, necrosis and yellowing. The other susceptible control, Haruna Nijo, displayed mosaicism and yellowing. The susceptible control Ko A displayed mild mosaicism when inoculated with BaMMV-Ka1. The other susceptible control, Haruna Nijo, displayed mosaic symptoms. Further, the reaction of the susceptible control Ko A to BaMMV-Na1, was faintly mosaic to mildly mosaic; and in the other susceptible control, Haruna Nijo, mosaicism was observed. The infection rates of the susceptible control Ko A were 100%, 94% and 71%, and in the other susceptible control, Haruna Nijo, were 63%, 87% and 72%, respectively. On the other hand, the resistant control Mokusekko 3 did not show any disease symptoms. The *rym1* gene-carrying lines also did not show any disease symptoms, which was the same as the level of resistance shown by Mokusekko 3 (Fig. 4). Further, the absence of the *rym1* gene in the BC_2F_3 lines showed the same reaction as Haruna Nijo, and the infection rates were 58%, 90% and 72%, respectively.

Linkage analysis of *rym1* on chromosome 4H

Linkage relationships between the reaction to BaYMV-I and the three DNA markers and one morphological marker on chromosome 4H were indicated. In the F_2 population of Y4 × Haruna Nijo, segregation of three DNA markers on chromosome 4H fits a 1:2:1 ratio of aa (homozygous for resistance): Aa (heterozygous for susceptibility): AA (homozygous for susceptibility): χ^2 for MWG2134 = 2.34 (P = 0.31), χ^2 for MWG2159 = 2.35 (P = 0.31), χ^2 for MWG058 = 3.97 (P = 0.14). Further, the segregation of the morphological marker *Bl* was investigated using the BC_1F_1 population of Y4 × Haruna Nijo in the breeding process. The result of the segregation of MWG2134 and *Bl* did not fit a 1:1 ratio of the BC_1F_1 population. This indicates that they were linked on chromosome 4H. Recombination values were estimated to be between the DNA markers and *rym1* on chromosome 4H, and are indicated in Table 4. It can be inferred from previous reports (Carlsberg Research Communications 1987; Konishi et al. 1997; Miyazaki et al. 2001), and the results of this study, that the *rym1* locus and the *Bl* locus were linked on chromosome 4H. A linkage map of chromosome 4H containing MWG2134, MWG2159, MWG058, *Bl* and *rym1* is illustrated in Fig. 5.

Discussion

In this study, only *rym1* was introgressed into the modern barley cultivar using RFLP markers, and the effect of the *rym1* gene on the infection by the BaYMV and BaMMV strains was then investigated. The above results indicated that *rym1* was completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1, and had an acceptable level of resistance to BaYMV-III. There are two interpretations of the *rym1* reaction to BaYMV-III. Mokusekko 3 is completely resistant to all the strains of BaYMV and BaMMV in Japan (Kashiwazaki et al. 1989; Iida et al. 1992; Nomura et al. 1996) and was carrying at least two resistance genes, *rym1* and *rym5* (Konishi et al. 1997). One interpretation of the reaction of BaYMV-III to Mokusekko 3 is the incidence of multiplicative gene action. Previous studies (Kashiwazaki et al. 1989; Iida et al. 1992; Nomura et al. 1996; Konishi et al. 1997) and the present study, inferred that the combination of *rym1* and *rym5* showed complete resistance to BaYMV-III infection. On the other hand, Miyazaki et al. (2001) recently reported that three QTLs were detected from Mokusekko 3 for BaYMV resistance. One of the QTLs was located on chromosome 4H for *rym1*, whilst *rym5* was located on chromosome 3HL. In addition to two QTLs, a new QTL for BaYMV resistance was identified in the terminal region of chromosome 7HS. Thus, there is the possibility that the new QTL had an effect on BaYMV-III. However, the result of this investigation of the DNA markers linked to the new QTL revealed segregation in the F_3 assessment lines (data not shown). Therefore, it seems reasonable to conclude that Mokusekko 3 acquired complete resistance to BaYMV-III by the multiplicative gene action of *rym1* and *rym5*. However, further investigation is now required. In conclusion, the *rym1* gene does confer unproblematic resistance to all

the Japanese strains of BaYMV and BaMMV. This study is the first report of the effect of the *rym1* gene's resistance to BaYMV-I, -II, -III, BaMMV-Ka1 and -Na1.

Bauer et al. (1997) reported that the BaMMV resistance-gene *ym11* originating from a Russian-barley landrace, Russia 57, is tightly linked to the RFLP marker MWG2134 on chromosome 4H, and that *ym11* confers resistance to all the European strains of BaYMV and BaMMV. In the present study, the gene *rym1*, which is resistant to Japanese BaYMV-I, -II, -III and BaMMV-Ka1, -Na1 strains, was also mapped near MWG2134. It is known that the resistance genes are not equally distributed among the chromosomes, but tend to form clusters in specific chromosomal regions. Different resistance genes in the same cluster often display different race specificities among the same pathogen species (Hammond-Kosack and Jones 1997). Further analysis is required to determine whether *rym1* is allelic to *ym11* from Russia 57, or whether the two genes are located at different resistance loci in the same cluster.

In Japan, using Mokusekko 3 as a cross parent, many BaYMV-resistant malting barley cultivars have been developed. Though Mokusekko 3 harbors at least two BaYMV resistance genes, only *rym5* was used for barley breeding against BaYMV. As described above, in the breeding programs for BaYMV resistance, *rym1* was not commonly introgressed into the established cultivars. The question arises why the important resistance gene *rym1* was eliminated in common with resistant cultivars during the breeding for resistance to BaYMV. Assuming a close linkage between *rym1* and an unfavorable QTL for malting barley, *rym1* could be easily dropped by eliminating the QTL. The malting quality was also investigated using BC_1F_4 carrying a *rym1* gene derived from Y4×Haruna Nijo, backcrossed with Haruna Nijo and selected using DNA markers. As a result, there were no significant differences between BC_1F_4 lines and Haruna Nijo (data not shown). On the other hand, the elimination of *rym1* might be due to the linkage drag between *rym1* and QTLs with unfavorable agronomic characteristics being derived from Mokusekko 3. Previous analysis revealed that the *rym1* locus was located on chromosome 4H and was linked to the morphological markers *K* and *glf3* with a recombination value of 25.3% and 9.7% (Konishi et al. 1997). In this present investigation, the *rym1* locus was found to be located on chromosome 4H and most tightly linked to the RFLP marker MWG2134 with a recombinant value of 4.17%, while MWG2134 was linked to the morphological marker *Bl* with a recombinant value of 8.42% (Table 4 and Fig. 5). Further, it is likely that the *rym1* locus and the *Bl* locus were linked with recombinant values of about 4.25% to 12.59%. These results suggested that the *rym1* locus and the *Bl* locus were tightly linked on chromosome 4H. Given that these results are accurate, the elimination of *rym1* might be due to the linkage drag between *rym1* and the unfavorable agronomic character *Bl* derived from Mokusekko 3. In Japan, the blue aleurone tends to be regarded as an unfavorable character and was eliminated in the malting barley breeding program. In consequence, it might be inferred from these results that in past breeding programs for BaYMV resistance, *rym1* was not introgressed into the established cultivars. This study has already broken the tightly linked *rym1* and *Bl*, and is currently breeding a new variety of malting barley carrying only the *rym1* gene resistant to all Japanese strains of BaYMV and BaMMV. In addition, a combination of *rym1*, *rym3* and *rym5* is now being attempted using DNA markers and field infection. Molecular marker-assisted selection may further contribute to the introduction of a resistance gene, as well as combining resistance genes into a cultivar to stabilize resistance.

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