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Genetic analysis of disease resistance to all strains of BaYMV in a Chinese barley landrace, Mokusekko 3

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Abstract A Chinese landrace of barley, Mokusekko 3, is unique in being completely resistant against all strains of barley yellow mosaic virus (BaYMV). The present investigation revealed that the resistance of Mokusekko 3 is governed by two recessive genes. As one of the resistance genes was known to be tightly linked with alleles at the *Est* complex locus, consisting of the *Est1*, *Est2* and *Est4* loci for esterase isozymes, each of the resistance genes could be separated by means of marker-assisted selection using an isozyme allelic combination as a marker. One of the resistance genes, *ym1*, is linked to *K* (hooded lemma) and *gl3* (glossy leaf 3) with recombination values of 25.3% and 9.7% respectively, and these three genes are located in the order *K-gl3-ym1* on chromosome 4. Another newly designated resistance gene, *ym5*, is linked to alleles at the *Est* complex locus and *cu2* (curly growth 2), with recombination values of 1.9% and 19.5% respectively, in the order *cu2-Est-ym5* from proximal to distal on the long arm of chromosome 3. The complete resistance of Mokusekko 3 is caused by combining two resistance genes, *ym1* and *ym5*. However, almost all the "resistant" cultivars derived from crosses with Mokusekko 3 are susceptible to the recently detected strain BaYMV-III in Japan, since they contain only one resistance gene, *ym5*. Marker-assisted selection to combine resistance genes into a cultivar is discussed for the breeding of stabilizing resistance to BaYMV.

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Introduction

Barley yellow mosaic virus (BaYMV) causes one of the most serious diseases to malting barley in East Asia and winter barley in Europe. When barley seedlings are infected with BaYMV in the field the plants have yellow-mosaic and shrunken leaves, resulting in a reduction of plant growth and grain yield (Takahashi et al. 1973; Huth 1982; Friedt et al. 1985). Since BaYMV is transmitted by the soil-borne fungus *Polymyxa graminis* Led. (Toyama and Kusaba 1970), the best way to effectively and economically prevent plants from injury is to grow BaYMV-resistant cultivars.

For the breeding of resistant cultivars, barley germ plasm was extensively surveyed (e.g. Takahashi et al. 1973; Huth 1982; Friedt et al. 1985; Kawada 1991). As a result, the Chinese six-rowed landrace Mokusekko 3 was found to be completely resistant against both BaYMV and barley mild mosaic virus (BaMMV) in Japan and Europe (Takahashi et al. 1973; Kashiwazaki et al. 1989; Götz and Friedt 1993). After back- and multiple-crossings to Mokusekko 3, many BaYMV-resistant cultivars of two-rowed malting barley have been established in Japan: namely, 'Misato Golden', 'Nishino Gold', 'Kinuyutaka', 'Mikamo Golden', 'Tone Nijo', 'Nishino Chikara', 'Asaka Gold', 'Yachiho Golden', and 'Takaho Golden'.

Meanwhile, three strains of BaYMV have been isolated in Japan; designated I, II and III (Ogawa et al. 1987; Kashiwazaki et al. 1989; Usugi et al. 1989). Strain I is the most prevalent type throughout Japan, while strain II is distributed in the limited area where the six-rowed barley cultivar, 'Kashimamugi' is grown. Strain III has been detected in the field-grown 'Misato

Golden' and 'Mikamo Golden' which are "resistant" cultivars to strains I and II bred from crosses with Mokusekko 3 (Ogawa et al. 1987; Kashiwazaki et al. 1989; Iida et al. 1992). Although "resistant" cultivars are susceptible to strain III, Mokusekko 3 is still completely resistant to all strains of BaYMV. From these results, it is necessary to clarify the genetic difference in BaYMV resistance between Mokusekko 3 and the "resistant" cultivars.

Takahashi et al. (1973) demonstrated that Mokusekko 3 carries a partially dominant resistance gene *Ym* and an additional gene with a slight resistance effect, and showed that *Ym* is linked with *K* for hooded lemma on chromosome 4 with 29.37% recombination. Meanwhile, using "resistant" lines to introduce the BaYMV resistance gene from Mokusekko 3, Konishi et al. (1989) reported that the resistance locus is tightly linked with the complex locus for esterase isozymes (*Est1-Est2-Est4*, abbreviated to *Est*) at the terminal end of the long arm of chromosome 3 with recombination values ranging from 1.26 to 5.01%. Furthermore, Konishi and Kaiser (1991) examined the genetic difference in BaYMV resistance between Mokusekko 3 and one of the "resistant" cultivars, 'Misato Golden'. Both of them carry one common resistance gene linked closely to the *Est* complex locus on chromosome 3, and Mokusekko 3 further possesses an additional resistance gene linked loosely to *K* on chromosome 4. These results suggest that the resistance to all strains of BaYMV in Mokusekko 3 may be caused by a combination of at least two resistance genes.

In the present study, the objectives were to clarify the genetic constitution of BaYMV-resistance in Mokusekko 3, to examine the effects of individual resistance genes on the reaction to BaYMV strains, and to construct a linkage map of each resistance gene. Additionally, for the breeding of stabilizing resistance to all strains of BaYMV, marker-assisted selection to combine resistance genes into a single cultivar is discussed.

Materials and methods

Mokusekko 3 is resistant to the newly established strain III of BaYMV, whereas its derived "resistant" cultivars, including 'Misato Golden', are susceptible. For examining the genetic difference between these two genotypes, 167 F_3 progenies of Mokusekko 3 × 'Misato Golden' were grown in a field infected with strain III of BaYMV, and segregation of the reaction was investigated.

Since Mokusekko 3 contains at least two resistance genes to BaYMV (Takahashi et al. 1973; Konishi and Kaiser 1991), it is necessary to separate the individual resistance genes to conduct further genetic analysis. For this purpose, an F_2 population of Mokusekko 3 × Colsess (susceptible) was grown in a field infected with strain I of BaYMV, and plants which were thought to carry each of the resistance genes were indirectly selected by the esterase isozyme genotype as illustrated in Fig. 1. One of the resistance genes is tightly linked with alleles at the *Est* complex locus, and Mokusekko 3 has a *Ca-ne-Nz* genotype consisting of the allelic combination *Est1-Est2-Est4* for the esterase isozymes (Konishi and Kaiser 1991). A BaYMV-resistant plant of the *Ca-ne-Nz* genotype was selected, and its progeny (501-124) proved to be homozygous for resistance. A plant of progeny 501-124 was randomly selected and crossed with Choshiro-hen, a susceptible and multiple marker stock of chromosome 3, carrying *uz* for uzu or semi-brachytic, *cu2* for curly growth 2, and *Af-ne-Su* for the esterase isozyme genotype. A linkage relationship of the resistance gene to these markers on chromosome 3 was examined using 250 F_3 progenies of 501-124. Choshiro-hen grown in a field infected with strain I of BaYMV.

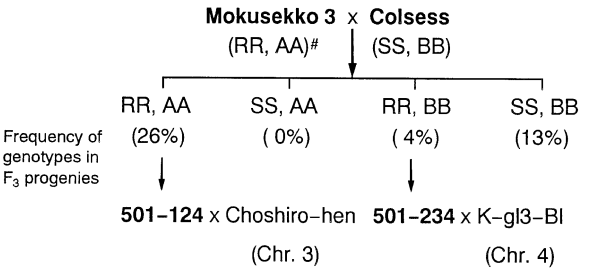

Fig. 1 Selection of F_3 progenies carrying each of the BaYMV resistance genes from Mokusekko 3 by esterase isozyme markers, and cross combinations for the linkage analysis of each resistance gene. # RR: resistant homozygote to BaYMV-I, SS: susceptible homozygote, AA: *Ca-ne-Nz* homozygote for esterase isozymes, BB: *Al-Fr-At* homozygote

Another resistance gene, *Ym*, of Mokusekko 3 is loosely linked to *K* for hooded lemma on chromosome 4 (Takahashi et al. 1973), showing that *Ym* is independent of *Est* on chromosome 3. In the same F_2 population of Mokusekko 3 × Colsess, several resistant segregants of the *Al-Fr-At* genotype with an esterase isozyme genotype the same as that of the susceptible parent, Colsess, were selected. They were either rare recombinants carrying the resistance gene closely linked with *Est* on chromosome 3, plants with only the *Ym* gene, or segregants possessing both resistance genes as in Mokusekko 3. F_3 progenies of these segregants were grown to identify homozygosity for resistance to BaYMV. As the *Ym* progenies had a high probability of appearing among these F_3 resistant offspring of the *Al-Fr-At* genotype (Konishi and Kaiser 1991), five progenies were randomly chosen, and one plant of each progeny was crossed with *K-gl3-Bl*, a susceptible and multiple marker stock of chromosome 4, containing *K* for hooded lemma, *gl3* for glossy leaf 3, *Bl* for blue aleurone, and *Hs* for hairy leaf sheath. Five F_2 populations were grown in a field infected with strain I of BaYMV, and segregation of the reaction to BaYMV was investigated in each population. The F_2 population of 501-234 × *K-gl3-Bl*, in which segregation of resistant vs susceptible plants fits a 1:3 ratio, was harvested individually, and 277 F_3 progenies were examined in terms of segregation for the reaction to strain I of BaYMV and the visible markers mentioned above. As the progeny of 501-234 carries only one resistance gene, *Ym*, isolated from Mokusekko 3, the effect of the *Ym* gene on the resistance to BaYMV strains I and III was further investigated using F_1 plants of 501-234 × susceptible lines, together with their parents. However, infection by BaYMV III was not sufficient for the reaction of these materials to be determined.

An allelism test was made between Japanese and German resistance genes tightly linked to the *Est* complex locus on chromosome 3, using 258 F_3 progenies derived from a cross between 'Misato Golden' and 'Sonate'. 'Sonate' is one of the German resistant cultivars to European BaYMV and BaMMV, carrying the resistance gene *ym4* closely linked to *Est* (Graner and Bauer 1993; Le Gouis et al. 1995).

Variations at the *Est1*, *Est2* and *Est4* loci of F_2 individuals were examined by a starch-gel electrophoresis, and their genotypes were expressed by allelic combination at the *Est1-Est2-Est4* loci (Nielsen and Johansen 1986; Konishi et al. 1989). Segregation of the reaction to BaYMV, as well as for visible markers, was determined in F_3 progenies, each of which consisted of about 30 plants. Strains I, II

Table 1 Interrelationship between resistance to BaYMV-III and two/six-rowed spike character in F_3 progenies of Mokusekko 3 (RR, *vv*) × 'Misato Golden' (*SS*, *VV*). Chi-square test for 1:2:1. χ^2 (BaYMV) = 3.76, $\chi^2(Vv) = 0.64$, χ^2_L (linkage) = 6.74

Two/six-rowed spike[b]	Reaction of BaYMV-III[a]			
	RR	RS	SS	Total
VV	9	17	13	39
Vv	31	39	12	82
vv	12	24	10	46
Total	52	80	35	167

[a] RR: resistant homozygote, RS: heterozygote for reaction, SS: susceptible homozygote
[b] *VV*: two-rowed homozygote, *Vv*: heterozygote for spike row, *vv*: six-rowed homozygote

Table 3 Reaction of parents and their F_1 plants to BaYMV-I

Parent and F_1 plant	Score of symptom	Reaction to BaYMV
Parent		
Mokusekko 3	0	R
501-234	0	R
Daisen Gold	5	S
New Golden	5	S
Svanhals	5	S
Misato Golden	0	R
F_1 plant		
501-234 × Daisen Gold	4.5	S
501-234 × New Golden	4.5	S
501-234 × Svanhals	4	S
Daisen Gold × Misato Golden	3	M

and III of BaYMV are abbreviated here as BaYMV-I, BaYMV-II and BaYMV-III, respectively.

Results

Difference between Mokusekko 3 and 'Misato Golden'

The segregation of the reaction to BaYMV-III in F_3 progenies of Mokusekko 3 × its derived "resistant" cultivar 'Misato Golden' fits a 1:2:1 ratio of RR (homozygous for resistance): RS (heterozygous for resistance): SS (homozygous for susceptibility) ($\chi^2 = 3.76$, $P = 0.10$–0.20), indicating that the difference in the reaction to BaYMV-III between Mokusekko 3 and 'Misato Golden' is governed by a single gene (Table 1). This shows that the susceptibility of 'Misato Golden' is caused by eliminating one of the resistance genes of Mokusekko 3 in the breeding of the cultivar. A chi-square test reveals that the resistance gene to BaYMV-III is independent of the two/six-rowed spike character controlling the *V*/*v* gene on chromosome 2.

Linkage analysis of *Ym* on chromosome 4

Segregation of the reaction to BaYMV-I in F_3 progenies of 501-234 × *K-gl3-Bl* was examined. As it was difficult to distinguish exactly between susceptible homozygous and heterozygous progenies, those progenies segregating suscepetible plants were treated as susceptible (S) as opposed to resistant homozygous ones (R) in the test for segregation. A chi-square test shows that the segregation fits a 1R:3S ratio ($\chi^2 = 3.96$, $P = 0.05$–0.10), suggesting that the resistance is controlled by a single gene (Table 2). Further, F_1 plants of 501-234 × susceptible lines were investigated in respect of their reaction to BaYMV-I. Although the parent of 501-234 was resistant to the virus, all of the F_1 plants were susceptible with the symptom scores of 4 and 4.5 showing very severe mosaic and yellowing which was close to the complete susceptiblity (score 5) of the susceptible parents (Table 3). This indicates that it is reasonable to consider the resistance of 501-234 to be governed by a recessive gene rather than a partially dominant one.

In the same F_3 progenies of 501-234 × *K-gl3-Bl*, segregation of three visible markers on chromosome 4 fits a ratio of 1:2:1: χ^2 for glossy leaf 3 (*gl3*) = 6.36 ($P = 0.05$–0.02); χ^2 for hooded lemma (*K*) = 3.96 ($P = 0.20$–0.50), χ^2 for hairy leaf sheath (*Hs*) = 0.96 ($P = 0.50$–0.90). However, it was difficult to distinguish white aleurone from light blue in the F_3 progenies, since the aleurone of the parent 501-234 was slightly bluish. The hairy leaf-sheath gene *Hs* is independent of

Table 2 Linkage data for the BaYMV resistance gene *ym1* and three markers on chromosome 4 in F_3 progenies of 501-234 × *K-gl3-Bl*. Chi-square test for 1:2:1. $\chi^2(gl3) = 6.36$*, $\chi^2(K) = 3.96$, $\chi^2(Hs) = 0.96$, χ^2(*ym1* for 3:1) = 3.96*

Gene pair		Segregation[a]	χ^2_L	Recombination value (%)
A	*B*			
gl3	*K*	6:27:34/26:108:23/30:20:3	69.88	24.18 ± 2.158
gl3	*Hs*	14:36:17/37:74:46/18:22:13	3.30	(Independent)
K	*Hs*	19:23:20/34:80:41/16:29:15	3.74	(Independent)
gl3	*ym1*	19:150:53/48:7:0	124.87	9.74 ± 1.859
K	*ym1*	57:135:30/5:20:30	35.45	25.28 ± 2.957
Hs	*ym1*	59:102:61/10:30:15	1.72	(Independent)

[a] *AABB*: *AABb*: *AAbb*/*AaBB*: *AaBb*: *Aabb*/*aaBB*: *aaBb*: *aabb* or *AAB_*: *AaB_*: *aaB_*/*AAbb*: *Aabb*: *aabb* (*B_*: susceptible, *bb*: resistant)

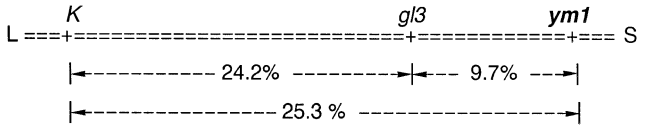

Fig. 2 A linkage map of chromosome 4 containing *ym1* for BaYMV resistance

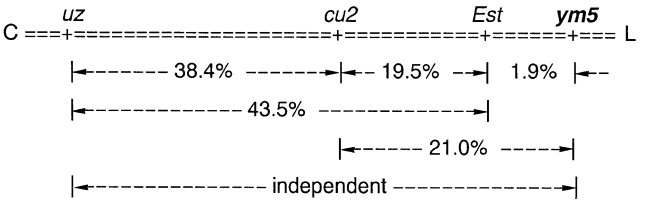

Fig. 3 A linkage map of the long arm of chromosome 3 containing *ym5* for BaYMV resistance

the above-mentioned marker genes on chromosome 4. Linkage relationships between the reaction to BaYMV-I and visible markers on chromosome 4 are indicated in Table 2. Recombination values were estimated to be 24.18% between *gl3* and *K*, 9.74% between *gl3* and the resistance gene, and 25.28% between *K* and the resistance gene. Takahashi et al. (1973) demonstrated that the resistance gene *Ym* is linked to *K* with a recombination value of 29.37 $\pm$ 3.559% on chromosome 4. This recombination value is not significantly different from the 25.28 $\pm$ 2.957% estimated in the present experiment.

From these results it may be concluded that the resistance gene of 501-234 derived from Mokusekko 3 is identical with *Ym*; however, as the resistance gene is recessive, it should be designated as *ym1* on chromosome 4, according to the recommended rules of the International Committee for nomenclature and gene symbolization in barley (1991). A linkage map of chromosome 4 containing *K*, *gl3* and *ym1* is illustrated in Fig. 2, and their loci are arranged in the order *K-gl3-ym5*.

Linkage analysis of the resistance gene on chromosome 3

Three loci for BaYMV resistance have been located on the long arm of chromosome 3, linked to the *Est* complex locus. They include the resistance gene of 'Misato Golden' derived from Mokusekko 3 (Konishi and Kaiser 1991), another resistance gene of 'Prior' to BaYMV-II (Iida and Konishi 1994), and the resistance gene *ym4* in the European BaYMV-resistant cultivars, 'Sonate', 'Franka', 'Diana' and others (Graner and Bauer 1993; Ordon et al. 1994).

A progeny of 501-124 which was resistant to BaYMV-I and possessed the same esterase isozyme genotype (*Ca-ne-Nz*) as Mokusekko 3 was crossed with Choshiro-hen, a susceptible and multiple marker stock of chromosome 3. F_3 progenies of 501-124 $\times$ Choshiro-hen were grown in a field infected with BaYMV-I, and segregation of the reaction to the virus was examined. Because of the difficulty in distinguishing accurately between susceptible homozygous and heterozygous progenies, these were pooled into a group of susceptible progenies. A chi-square test for the reaction indicates that the segregation fits a ratio of 1 (resistant):3 (susceptible) ($\chi^2 = 0.05$, $P = 0.50$–0.90) (Table 4), suggesting that the resistance of 501-124 derived from Mokusekko 3 is determined by a single gene. The segregation of esterase isozyme genotypes fits a 1:2:1 ratio, but segregation distortion was observed at the *uz* and *cu2* loci respectively. This distortion might be caused by a gametophytic gene such as *Ga2* (Konishi et al. 1990). Linkage analysis reveals that the four loci are located in the order *uz-cu2-Est*–the resistance gene, from proximal to distal, in the long arm of chromosome 3 (Fig. 3), and that the resistance gene is tightly linked to the esterase isozyme alleles at the *Est* complex locus with a recombination value of 1.93 $\pm$ 0.877%. This recombination value is not significantly different from the value of 2.45 $\pm$ 0.732% estimated from F_3 progenies of 'Misato Golden' $\times$ Colsess (Konishi and Kaiser 1991), indicating that the resistance gene of 501-124 is

Table 4 Linkage data between the BaYMV resistance gene *ym5* and three markers on chromosome 3 in F_3 progenies of 'Misato Golden' $\times$ Choshiro-hen. Chi-square test for 1:2:1. $\chi^2(Est) = 1.94$, $\chi^2(uz) = 29.20$**, $\chi^2(cu2) = 31.68$**, $\chi^2(ym5$ for $3:1) = 0.05$

Gene pair		Segregation[a]	χ^2_L	Recombination value (%)
A	*B*			
Est[b]	*uz*	27:31:7/47:72:13/16:27:10	4.56	43.54 $\pm$ 3.086
Est	*cu2*	53:12:0/42:78:12/6:11:36	148.28	19.54 $\pm$ 2.020
uz	*cu2*	43:39:8/48:52:30/10:10:10	22.23	38.43 $\pm$ 2.931
Est	*ym5*	3:130:53/62:2:0	234.54	1.93 $\pm$ 0.877
uz	*ym5*	62:100:24/28:30:6	2.99	(Independent)
cu2	*ym5*	46:92:48/55:9:0	99.03	21.04 $\pm$ 2.653

[a] See the footnote of Table 2
[b] Genotype at the *Est* complex loci *AA*: *Ca-ne-Nz*/*Ca-ne-Nz*, *AB*: *Ca-ne-Nz*/*Af-ne-Su*, *BB*: *Af-ne-Su*/*Af-ne-Su*

Table 5 Interrelationship between reaction to BaYMV-I and esterase isozyme genotypes in F_3 progenies of 'Misato Golden' (RR, *AA*) × 'Sonate' (RR, *BB*). Chi-square test χ^2(BaYMV for 7:9) = 4.49*, χ^2(*Est* for 1:2:1) = 0.05

Est[a]	Reaction to BaYMV-I		
	RR	RS + SS	Total
AA	41	25	66
AB	31	97	128
BB	24	40	64
Total	96	162	258

[a] *AA*: *Ca-ne-Nz*/*Ca-ne-Nz*, *AB*: *Ca-ne-Nz*/*Ca-Fr-Su*, *BB*: *Ca-Fr-Su*/*Ca-Fr-Su*

identical to that of 'Misato Golden', both of which were derived from Mokusekko 3.

As mentioned previously, the question still remains whether the resistance gene of 'Misato Golden' is allelic to *ym4* of 'Sonate'. F_3 progenies of 'Misato Golden' × 'Sonate' were grown in a field infected with BaYMV-I, and the segregation of the reaction to the virus as well as the interrelationship between the resistance gene and esterase genotype were examined. Segregation of the reaction to BaYMV was so complex as to suggest that intra- and inter-allelic interactions might exist (Table 5). However, susceptible individuals frequently appeared in the progenies, showing that the resistance gene of 'Misato Golden' differs from *ym4* of 'Sonate'. Furthermore, compared with RFLP map of *ym4* (Graner and Bauer 1993), the gene is located distal the *Est* complex locus, while *ym4* is proximal. As shown in Table 3, F_1 to plants of the susceptible cultivar 'Daisen Gold' × 'Misato Golden' were also susceptible, indicating that the resistance is controlled by a recessive gene. From these results, it can be safely concluded that the resistance gene of 'Misato Golden' and 501-124 is newly assigned to *ym5*, and that Mokusekko 3 carries both *ym1* and *ym5*. In addition, it should be noted that the gene *Ym*, linked with the *Est* complex locus as given in our previous reports (Konishi et al. 1989; Konishi and Kaiser 1991), should be amended to *ym5*.

Discussion

Compared with fungi and bacteria, breeding for resistance to the soil-borne virus BaYMV is difficult and troublesome for the following reasons; no artificial inoculation, transmission by a soil-inhabiting fungus, mixed infection with viruses showing similar symptoms, unstable infection affected by natural conditions, and irregular distribution of the virus in the infected field. These factors often lead to inconsistent results as a consequence of a failure of the infection. Furthermore, the virus consists of different strains showing interactive reactions to barley genotypes so that a new strain may easily appear. Three strains of BaYMV have been classified in Japan, and further divided into subgroups. In Europe, two strains of BaYMV and BaYMV-2 have been detected. Moreover, new strains of BaYMV appeared soon after the release of "BaYMV-resistant" cultivars to farmers. BaYMV-III was detected in areas growing "resistant" cultivars carrying *ym5*, such as 'Misato Golden' and 'Mikamo Golden' in Japan (Kashiwazaki et al. 1989), while BaYMV-2 was found in areas growing "resistant" cultivars with *ym4*; namely, 'Birigit', 'Franka' and 'Sonate' in Germany and 'Torrent' in England (Huth 1989). The recent determination of the sequence of numerous RNA viruses, including BaYMV, reveals that RNA recombination has played a part in the evolution of viruses (Simon and Bujarski 1994), suggesting that a new strain of the virus can easily and frequently develop. The occurrence of a new strain of BaYMV can be detected only when a "resistant" cultivar changes to susceptible.

Mokusekko 3 is completely resistant to all strains of BaYMV and BaMMV in Japan (Kashiwazaki et al. 1989; Iida et al. 1992) and Germany (Friedt et al. 1985; Huth 1991; Götz and Friedt 1993). Takahashi et al. (1973) surveyed a large number of barley varieties grown in BaYMV-infected fields, and found that Mokusekko 3 proved to be the most promising for disease resistance breeding. They also conducted genetic analysis of BaYMV resistance in Mokusekko 3, and demonstrated that this resistance is chiefly controlled by a partially dominant gene, *Ym*, which is linked with *K* for hooded lemma on chromosome 4, with 29.37% recombination, together with another gene which has a slight resistance effect. Since then, only a few genetic studies on the BaYMV resistance of Mokusekko 3 have been carried out, indicating that resistance is governed by two or more genes with allelic interactions (Table 6). One of the barriers to progress in the genetic analysis of the resistance was how to separate the individual resistance genes. The finding that one of the resistance genes is tightly linked with the *Est* alleles (Konishi et al. 1989; Konishi and Kaiser 1991) was effective in isolating *ym5* from the resistance genes of Mokusekko 3, so that the remaining resistance gene(s) could be analyzed independently of *ym5*. Fortunately, this involves only a single recessive gene, *ym1,* which is identical with the partially dominant gene *Ym* designated by Takahashi et al. (1973). The complete resistance to BaYMV and BaMMV of Mokusekko 3 is thus controlled by two recessive genes, *ym1* and *ym5*, although almost all the "resistant" cultivars in Japan and Europe possess one, resistance gene, *ym5* or *ym4* respectively.

For the breeding stabilizing resistance to BaYMV and BaMMV, it is necessay to combine two or more resistance genes into a single cultivar. Before discussing this problem, it is necessary to investigate the reaction to BaYMV strains of Japanese "BaYMV-resistant"

Table 6 Segregation of the reaction to BaYMV in F_2 populations or F_3 progenies of Mokusekko 3 × susceptible cultivar or tester stocks

Mokusekko 3 crossed with		Segregation[a]			χ^2		Author
		Resist.	Suscept.	Total	(1R : 3S)	(7R : 9S)	
Colsess IV	F_2	71	177	248	1.74	23.04**	Takahashi et al. (1973)
Colsess Ia	F_3	100	174	274	19.31**	5.86*	Konishi and Kaiser (1991)
Haruna Nijo	F_2	84	155	239	13.12**	7.19**	Sohtome et al. (1991)
Choshiro-hen	F_3	99	189	288	13.58**	10.29**	Konishi et al. (unpublised)

[a] F_3 data were converted into F_2 segregation
* and **: significant at the 5 and 1% level, respectively

cultivars and lines bred from cross combinations with Mokusekko 3. Of 39 "resistant" cultivars and lines examined, all of them were susceptible to BaYMV-III with only one exceptional line which like Mokusekko 3 was resistant (Iida 1990). The susceptible ones, including "resistant" cultivars such as 'Misato Golden', 'Nishino Gold' and 'Mikamo Golden', carry only the *ym5* resistance gene. The difference between genotypes resistant or susceptible to BaYMV-III may be caused by the presence or absence of the additional resistance gene *ym1*. The question arises why the important resistance gene *ym1* was eliminated in common with "resistant" cultivars during the breeding for resistance to BaYMV. Assuming a close linkage between *ym1* and an unfavorable QTL for malting barley, *ym1* could be easily dropped by eliminating the QTL. Further investigation is required to clarify the unfavorable QTL linked to *ym1* and the possiblity of breaking the tight linkage between both loci.

It is also important to establish a suitable procedure to combine two or more resistance genes into a single cultivar through marker-assisted selection (MAS), since selection in the field has been laborious and of low efficiency. Indirect selection of the *ym5* genotype involving the *Ca-ne-Nz* genotype at the *Est* complex locus is both practicable and efficient, because selection can be carried on at the seedling stage in the laboratory. There are several BaYMV-resistance genes to be combined with *ym5*. Among them, *ym4* of German resistant cultivars and the resistance gene to BaYMV-II of 'Prior' (Iida and Konishi 1994) are linked to the *Est* complex locus respectively, so MAS to combine either of these resistance genes with *ym5* can be carried out by means of using the esterase isozyme genotype as a marker. For instance, plants carrying the same *Ca-ne-Nz* genotype for esterase isozymes as that of Mokusekko 3 selected from crosses with Mokusekko 3 have a high probability of possessing *ym5*. In addition, *ym4* is closely linked with an RFLP marker, MWG 10 (Graner and Bauer 1993), and a RAPD marker, OP-Z04 (Ordon et al. 1994). Therefore, esterase and molecular markers are effective in selecting plants carrying both *ym4* and *ym5* resistance genes from a hybrid population of 'Franka' (*ym4*) × 'Misato Golden' (*ym5*). However, it should be noted that 'Sonate' and 'Diana' carrying *ym4* are susceptible to BaYMV-III, although they are resistant to BaYMV-I and BaYMV-II in Japan (Iida 1990). Another gene resistant to all strains of BaYMV is *ym3*. The *ym3* gene is found in 'Haganemugi', 'Ishuku Shirazu' and 'Ea 52' (Ukai and Yamashita 1980; Kawada 1991), and expresses resistance to all strains of BaYMV in Japan. However, *ym3* is not effective against BaMMV, and has not been localized on any chromosome. When a suitable marker tightly linked to *ym3* is found, a combination between *ym3* and *ym5* may result in achieving stabilizing resistance to BaYMV. The alternative is to combine *ym1* and *ym5* as in Mokusekko 3. Compared with molecular markers, isozyme markers have several advantages for MAS in crop breeding (Konishi 1995). Therefore, it is the first step to be employed in determining linkage between a resistance gene and an isozyme allele. If this is not possible, a molecular marker closely linked to the resistance gene can be used in the next step (Konishi 1996). Molecular marker-assisted selection may further contribute to the introduction of a resistance gene as well as combining resistance genes into a cultivar for stabilizing resistance.

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