Interaction between Barley Yellow Mosaic Disease-resistance Genes *rym1* and *rym5*, in the Response to BaYMV Strains

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A Chinese landrace of barley, Mokusekko 3, is completely resistant to all the present strains of Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV), and has at least two resistance genes, *rym1* and *rym5*. However, *rym1* has not been used for BaYMV-resistance breeding of barley in Japan so far. In our previous study, *rym1* was completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1, and was acceptably resistant to BaYMV-III. Complete resistance of Mokusekko 3 was estimated to be due to the interaction between *rym1* and *rym5*. To estimate pyramiding effects of these genes using DNA markers, we investigated the resistance reaction of lines with or without these genes to BaYMV-I and -III. All the pyramiding lines showed complete resistance to BaYMV-I and -III. This study anticipated the resistance to BaYMV-III of Mokusekko 3 by the multiple effect of the *rym1* and *rym5* genes.

Key Words: resistance gene, pyramiding, marker assisted selection, virus resistance, barley yellow mosaic virus, barley mild mosaic virus.

Introduction

East Asian and European winter barley cultivars are seriously damaged by BaYMV and BaMMV (Ikata 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 severe symptoms such as yellow streaking and brown necrotic patches. In Japan, BaYMV occurrence is widespread, and the virus causes serious damage to two-rowed malting barley (Kashiwazaki *et al.* 1990). Based on virulence tests on different barley cultivars, the BaYMs 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 identified (Nomura *et al.* 1996).

The most common approach in preventing 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 present 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 released. Although Mokusekko 3 harbors at least two BaYMV resistance genes, only *rym5* has been used for barley breeding against BaYMV. The *rym5* gene was resistant to BaYMV-I, -II and BaMMV-Ka1 in Japan (Kashiwazaki *et al.* 1989, Iida *et al.* 1992), but susceptible to BaYMV-III (Ogawa *et al.* 1987). Another gene that is 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, but is not effective on BaMMV. In our previous study (Okada *et al.* 2003a), *rym1* was completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1. It was partially and acceptably resistant to BaYMV-III. Chromosomal locations of *rym1* (Miyazaki *et al.* 2001) and *rym5* (Graner *et al.* 1995, 1999, Miyazaki *et al.* 2001) on RFLP linkage map have been identified. A new (7HS) QTL for BaYMV resistance was also identified in Mokusekko 3 (Miyazaki *et al.* 2001). This study focuses on the resistance of Mokusekko 3 by interaction between *rym1* and *rym5*, performed the pyramiding of these genes, and investigated the resistance to BaYMV-I and -III, followed by the discussion on the 7HS QTL for BaYMV resistance derived from Mokusekko 3.

Materials and Methods

Plant materials

rym1 donor: A BaYMV-resistant F₄ progeny, derived from Ko A × Mokusekko 3, carrying only *rym1*, was screened by DNA markers linked to *rym1* (MWG2134 and MWG2159) and linked to *rym5* (ABC172). An F₄ line, Y4,

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carrying only *rym1* isolated from Mokusekko 3 proved homozygous for resistance. A cross was made between Y4 as the female parent and Haruna Nijo as the male parent at the Bioresources Research and Development Laboratories (BRDL), Sapporo Breweries Ltd., Gunma, Japan, in 1999. Haruna Nijo released from Sapporo Breweries Ltd., is a Japanese malting variety with high malting and brewing quality profiles. However, it is susceptible to BaYMV and BaMMV. F₁ plants were grown in a summer nursery and then backcrossed with Haruna Nijo. The selection was made from 321 BC₁F₁ plants using DNA markers. After the marker genotyping of BC₁F₁, heterozygotes were selected and backcrossed with Haruna Nijo for two generations. The selected heterozygotes were selfed, and a DNA marker selection of progeny homozygous for *rym1* was made from BC₃F₂. These selections were carried out from the BC₁F₁ to BC₃F₂ generations (Mokkei 02530) at BRDL up to 2001. The selected line Mokkei 02530 was investigated its graphical marker genotypes (58 loci), where all markers showed Haruna Nijo alleles (Fig. 1).

rym5 donor: The malting barley line Nittakei 62 was bred in BRDL by cross breeding method having a source of *rym5* from Mokusekko 3 in its pedigree (Fig. 2). Nittakei 62 is a Japanese malting barley line with ordinary high malting quality as Haruna Nijo and carrying only *rym5*, resistant to BaYMV-I as Mokusekko 3. However, it is susceptible to BaYMV-III.

Pyramiding of resistance genes rym1 and rym5: A cross was made between Mokkei 02530 (*rym1*) and Nittakei 62 (*rym5*) at BRDL, Sapporo Breweries Ltd., Gunma, Japan,

in 2001. F₁ plants were grown in a summer nursery, and F₂ plants were grown in the field at BRDL. The first screening was made from 351 F₂ plants using CAPS markers (Okada *et al.* 2003c). The second selection was performed by southern hybridization. At the second selection, pyramiding types (*rym1* and *rym5*) were selected, and F₃ seeds were harvested. We obtained nine pyramiding lines in this cross, and the seven F₃ lines with sufficient seed samples were used for subsequent experiments (Fig. 3).

DNA extraction and first screening by CAPS

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). Cleaved Amplified Polymorphic Sequences (CAPS) analysis following the method in our previous report (Okada *et al.* 2003c) was conducted. This CAPS marker was developed from a restriction fragment length polymorphism (RFLP) marker probe, MWG2134, of barley, and it is a reliable PCR-based marker that can detect the resistance allele at the *rym1* gene locus.

Second screening by southern hybridization

Three µg of each DNA sample was individually digested with *Bam*HI or *Dra*I. These digested DNAs were electrophoresed on a 0.8% agarose gel and transferred to nylon membranes using 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.

Fig. 1. The status of graphical genotype markers in Mokkei 02530. Normal black markers indicated Haruna Nijo alleles. Markers shown in gray are Mokusekko 3 alleles.

Fig. 2. Pedigree of Nittakei 62 shows that the BaYMV-I resistance of Nittakei62 is depend on *rym5* of Mokusekko 3 origin.

Fig. 3. Molecular maps of the *rym5* (3HL: left) and *rym1* (4HL: right) resistance region based on the F₂ population of Ko A × Mokusekko 3. A skeletal map of Ko A × Mokusekko 3 (cf. Miyazaki *et al.* 2000) was used as a reference. Results from RFLP analysis are presented for linked markers to the resistance, indicated by bold lines: lane1; Amagi Nijo, lane2; Haruna Nijo, lane3; Mikamo Golden, lane4; Nittakei 62, lane5; Mokkei 02530, lane6; Mokusekko 3, lane7; SA2-5, lane8; SA2-15, lane9; SA2-18, lane10; SA2-43, lane11; SA2-63, lane12; SA4-9, lane13; SA4-29, lane14; SA2-19, lane15; SA2-25, lane16; SA4-6, lane17; SA4-27, lane18; SA2-22, lane19; SA2-29, lane20; SA4-23.

Investigation of resistance to BaYMV-I and -III

The F₃ lines were grown in a field infected only with BaYMV-I, and reaction was investigated in the 2002–2003 season. Parents of the cross, resistant/susceptible checks and differential genotypes were planted at the same time. Agronomic characters were individually examined. Disease reaction was evaluated individually based on mosaic symptoms and yellowing 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, homozygotes for *rym1* were selected using DNA markers. In the 2002–2003 season, 40 seedlings per F₃ line were grown in a field infected only with BaYMV-III and investigated their reaction to BaYMV-III. The assessment of resistance to BaYMV-III was also based on mosaic symptoms and yellowing on the leaves. The symptoms and yellowing were scored as 0.0 (none) to 6.0 (severe). All assessed plants were individually harvested and their agronomic characters were investigated.

7HS QTL region analysis

A QTL for BaYMV resistance was reported on the terminal region of chromosome 7HS (JBG235-JBG209) from Mokusekko 3 (Miyazaki *et al.* 2001). We analyzed this region on pyramiding lines using RFLP methods. Next, the relation between the 7HS region and BaYMV-III resistance on pyramiding lines were examined.

Results

The infection of barley seedlings by BaYMV-I or -III in the field was so effective that the resistant plants were easily distinguishable from the susceptible ones. The seedlings of the susceptible controls, Amagi Nijo and Haruna Nijo were entirely infected with BaYMV-I. However, the cultivar/line carrying the resistance genes (*rym1* and/or *rym5*) was not infected with BaYMV-I (Table 1). The susceptible controls, Amagi Nijo and Haruna Nijo displayed severe mosaic symptoms (symptom scores of 6.0 and 5.5), and severe yellowing (yellowing leaf scores of 5.5 and 4.0), in the BaYMV-III-infected field. The *rym5*-carrying cultivar/line, Mikamo Golden and Nittakei 62 also displayed mosaic symptoms (symptom scores of 5.5 and 5.0) and yellowing (yellowing leaf scores of 4.0). A resistant breeding line, Mokkei 02530 that carries only *rym1*, showed mosaics (symptom score of 4.0), similar to the susceptible cultivars. However, its yellowing score was 1.0, indicating mild yellowing. Other lines carrying only *rym1* also showed the same level of resistance to BaYMV-III. On the other hand, the completely resistant control, Mokusekko 3 showed neither any disease symptoms (symptom score of 0.0) nor yellowing (yellowing leaf score of 0.0). In addition, none of the pyramiding lines showed any disease symptoms nor yellowing (Table 1 and Fig. 4), which expressed the same resistance level as Mokusekko 3.

The agronomic characters, such as, culm length, spike

number, spike length and number of grains per spike of pyramiding lines did not significantly differ from the susceptible Haruna Nijo grown in BaYMV-I or -III infected field, except for the culm length. The susceptible Haruna Nijo was dwarfed (Table 2 and Fig. 5). On the other hand, culm length was not different between pyramiding lines and Haruna Nijo grown in uninfected field (Table 2).

Furthermore, the investigation of the DNA marker was carried out about the 7HS QTL region (JBG235-JBG209).

Fig. 4. Effect of the combination of *rym1* and *rym5* genes under the infection of BaYMV-III. A and B; Reaction to BaYMV-III with severe mosaic and yellowing in Mikamo Golden with *rym5* (A) and Haruna Nijo (B). C; Reaction to BaYMV-III with severe mosaic symptoms and very mild yellowing in Mokkei 02530 with the *rym1*. D; Reaction to BaYMV-III in the pyramiding line without symptoms and yellowing with *rym1* and *rym5*.

Fig. 5. Barley plants grown in a field infected with BaYMV-III. The susceptible cultivar Haruna Nijo and *rym5*-carrying lines were dwarfed by the infection. Numbers indicate the genotypes as 1; *rym1*-carrying line Mokkei 02530, 2; *rym1* and *rym5* carrying pyramiding line, 3; susceptible control, Haruna Nijo, 4; *rym5*-carrying line Nittakei 62.

The QTL of 7HS originally detected in Mokusekko 3 differed from Nittakei 62 and Mokkei 02530 in the banding pattern of the marker JBG209. On the other hand, Mokusekko 3 and Mokkei 02530 showed the same banding pattern in another marker (JBG235) (Fig. 6). This QTL was not transferred from Mokusekko 3 to the Nittakei 62, but Mokkei 02530 might have a Mokusekko 3 allele. These DNA markers were segregated among the assessed pyramiding lines, which showing complete resistance (Fig. 6).

Discussion

In previous study, we investigated the effect of *rym1* gene on infection with BaYMV and BaMMV strains. The above results indicated that *rym1* is completely resistant to BaYMV-I, -II, BaMMV-Ka1 and -Na1, and has an acceptable level of resistance to BaYMV-III (Okada *et al.* 2003a). The *rym1* lines didn't show a completely resistance to BaYMV-III like Mokusekko 3. 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.*

Fig. 6. A molecular map of the 7HS QTL region on the F_2 population of Ko A $\times$ Mokusekko 3 based on the skeletal map (cf. Miyazaki *et al.* 2000). Results from RFLP analysis are presented for selected markers, indicated by bold lines: lane1; Amagi Nijo, lane2; Haruna Nijo, lane3; Mikamo Golden, lane4; Nittakei 62, lane5; Mokkei 02530, lane6; Mokusekko 3, lane7; SA2-5, lane8; SA2-15, lane9; SA2-18, lane10; SA2-43, lane11; SA2-63, lane12; SA4-9, lane13; SA4-29.

1996) and it carries at least two resistance genes, *rym1* and *rym5* (Konishi *et al.* 1997). In addition, Miyazaki *et al.* (2001) detected a 7HS QTL with additional effect on BaYMV resistance in Mokusekko 3, but this QTL was not transferred from Mokusekko 3 to the resistance donor parents used in this study. The DNA markers linked to the 7HS QTL segregated in the assessed BaYMV-III resistant F_3 lines having *rym1* and *rym5* (Fig. 6). Our results suggest that the qualitative resistance of 7HS QTL was not high enough to effect the resistance expression to *rym1* and *rym5*. It is concluded that Mokusekko 3 acquired complete resistance to BaYMV-III mostly by multiple gene action of *rym1* and *rym5*. Recently, Konishi *et al.* (2002) reported that Mokusekko 3 showed complete resistance to the BaYMV-1, BaYMV-2 and BaMMV in Germany. These results suggest that Mokusekko 3 shows complete resistance in all present strains of BaYMV and BaMMV in Japan and Germany, and this can be explained by multiple gene action and/or interaction of *rym1* and *rym5*.

In Japan, using Mokusekko 3 as a cross parent, many BaYMV-resistant malting barley cultivars have been

Table 1. Reaction of varieties and lines to BaYMV-I and -III

Variety/Line	Resistance gene	Reaction to BaYMV-I	Reaction to BaYMV-III		
		R/S ¹⁾	Score of Mosaic	Score of Yellowing	R/S ¹⁾
Amagi Nijo	—	S	6.0	5.5	S
Haruna Nijo	—	S	5.5	4.0	S
Mikamo Golden	<i>rym5</i>	R	5.5	4.0	S
Mokusekko 3	<i>rym1, rym5</i>	R	0.0	0.0	R
Nittakei 62	<i>rym5</i>	R	5.0	4.0	S
Mokkei 98261	<i>rym5</i>	R	4.0	3.0	S
Mokkei 02530	<i>rym1</i>	R	4.0	1.0	AR
SA2-5	<i>rym1, rym5</i>	R	0.0	0.0	R
SA2-15	<i>rym1, rym5</i>	R	0.0	0.0	R
SA2-18	<i>rym1, rym5</i>	R	0.0	0.0	R
SA2-43	<i>rym1, rym5</i>	R	0.0	0.0	R
SA2-63	<i>rym1, rym5</i>	R	0.0	0.0	R
SA4-9	<i>rym1, rym5</i>	R	0.0	0.0	R
SA4-29	<i>rym1, rym5</i>	R	0.0	0.0	R
SA2-19	<i>rym1</i>	R	3.0	1.0	AR
SA2-25	<i>rym1</i>	R	5.0	2.0	AR
SA4-6	<i>rym1</i>	R	4.0	1.5	AR
SA4-27	<i>rym1</i>	R	4.0	1.0	AR
SA2-22	<i>rym5</i>	R	5.5	4.0	S
SA2-29	<i>rym5</i>	R	5.5	3.0	S
SA4-23	<i>rym5</i>	R	6.0	4.0	S

¹⁾ R, resistant; S, susceptible; AR, acceptably resistant

Table 2. Agronomic characters of pyramiding lines in comparison with susceptible control 'Haruna Nijo', grown in an unfested and infested field by BaYMV-I or -III

Item	Variety/Line	Resistance gene	Culm length (cm)
Grown in an unfested field	Haruna Nijo	—	98.6±1.9
	SA2-15	<i>rym1, rym5</i>	100.8±4.9
	SA2-18	<i>rym1, rym5</i>	104.0±2.9
	SA2-43	<i>rym1, rym5</i>	101.8±2.5
	SA2-63	<i>rym1, rym5</i>	110.6±2.7
	SA4-9	<i>rym1, rym5</i>	95.6±3.4
	SA4-29	<i>rym1, rym5</i>	94.1±2.1
Grown in an infested field by BaYMV-I	Haruna Nijo	—	71.2±4.3
	SA2-15	<i>rym1, rym5</i>	97.0±1.9**
	SA2-18	<i>rym1, rym5</i>	101.0±2.6**
	SA2-43	<i>rym1, rym5</i>	98.8±2.2**
	SA2-63	<i>rym1, rym5</i>	104.4±3.0**
	SA4-9	<i>rym1, rym5</i>	94.4±3.1**
	SA4-29	<i>rym1, rym5</i>	97.7±2.9**
Grown in an infested field by BaYMV-III	Haruna Nijo	—	76.6±3.1
	SA2-15	<i>rym1, rym5</i>	96.0±6.8**
	SA2-18	<i>rym1, rym5</i>	99.3±9.7**
	SA2-43	<i>rym1, rym5</i>	102.0±3.0**
	SA2-63	<i>rym1, rym5</i>	89.6±5.6**
	SA4-9	<i>rym1, rym5</i>	99.1±6.9**
	SA4-29	<i>rym1, rym5</i>	98.1±4.9**

** = significant at the 0.01 probability level

released. Although Mokusekko 3 harbors at least two BaYMV resistance genes, only *rym5* was used for barley breeding against BaYMV and *rym1* was not commonly introgressed

into the established cultivars. It may be because there is a close link with *rym1* and blue aleurone (*Bl*), as indicated in our previous study (Okada *et al.* 2003a). We have already

broken a linkage between *rym1* and *Bl*, and developed a new malting barley line carrying *rym1* without *Bl* (Okada *et al.* 2003b). Recently, in Japan, barley yellow mosaic disease has been extended by the infection of BaYMV-III. A resistance gene to BaYMV-III, the *rym3* is used as a source of resistance in Japan. Genetic vulnerability or breakdown of resistance is a major concern in disease resistance breeding of barley if only the single source of resistance is used. In future breeding program, a combination of more than two resistance genes should be conducted. Molecular marker-assisted selection may further contribute to introducing a resistance gene, as well as resistance genes into a given genetic background to stabilize resistance.

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Literature Cited

- Graner, A., E. Bauer, A. Kellermann, G. Proeseler, G. Wenzel and F. Ordon (1995) RFLP analysis of resistance to the barley yellow mosaic virus complex. *Agronomie* 15: 475-479.
- Graner, A., S. Streng, A. Kellermann, A. Schiemann, E. Bauer, R. Waugh, B. Pellio and F. Ordon (1999) Molecular mapping and genetic fine-structure of the *rym5* locus encoding resistance to different strains of the Barley Yellow Mosaic Virus Complex. *Theor. Appl. Genet.* 98: 285-290.
- Huth, W. (1989) Ein weiterer Stamm des barley yellow mosaic virus aufgefunden. *Nachrichtenbl. Dtsch. Pflanzenschutzdienst* 41: 6-7.
- Huth, W. and D.E. Lesemann (1978) Ein für die Bundesrepublik neue Virose an Wintergerste. *Nachrichtenbl. Dtsch. Pflanzenschutzdienst* 30: 184-185.
- Iida, Y., K. Watanabe, I. Toshima and K. Ogawa (1992) Reaction of barley (*Hordeum vulgare* L.) cultivars and lines to barley yellow mosaic virus strains. *Jpn. J. Breed.* 42: 863-877.
- Iketa, S. and I. Kawai (1940) Studies on wheat yellow mosaic disease. *Noji Kairyō Shiryo* 154: 1-123.
- Kashiwazaki, S., K. Ogawa, T. Usugi, T. Omura and T. Tsuchizaki (1989) Characterization of several strains of barley yellow mosaic virus. *Ann. Phytopathol. Soc. Jpn.* 55: 16-25.
- Kashiwazaki, S., K. Nomura, K. Watanabe, I. Toshima, Y. Iida, T. Usugi, K. Ogawa, H. Hibino and T. Tsuchizaki (1990) Barley yellow mosaic virus and barley mild mosaic virus: strains and host resistance. In "Proc. 1st Symp. Int. Working Group Plant Viruses with Fungal Vectors" Koenig, R. (ed.), Baunschweig, p. 105-108.
- 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. Agri. Exp. Stn.* 27: 65-79.
- Konishi, T., T. Ban, Y. Iida and R. Yoshimi (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., F. Ordon and M. Furusho (2002) Reactions of barley accessions carrying different *rym* genes to BaYMV and BaMMV in Japan and Germany. *Barley Genet. Newslett.* 32: 46-48.
- Miyazaki, C., E. Osanai, K. Saeki, N. Hirota, K. Ito, T. Konishi and A. Saito (2000) A barley linkage map using an F₂ population compared with a map based on female recombination-derived doubled haploid lines. *Breed. Sci.* 50: 241-250.
- Miyazaki, C., E. Osanai, K. Saeki, K. Ito, T. Konishi, K. Sato and A. Saito (2001) Mapping of quantitative trait loci conferring resistance to barley yellow mosaic virus in a Chinese barley landrace Mokusekko 3. *Breed. Sci.* 51: 171-177.
- Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8: 4321-4325.
- Nomura, K., S. Kashiwazaki, H. Hibino, T. Inoue, E. Nakata, Y. Tsuzaki and S. Okuyama (1996) Biological and serological properties of strains of barley mild mosaic virus. *J. Phytopathol.* 144: 103-107.
- Ogawa, K., K. Watanabe, Y. Iida, I. Toshima, S. Kashiwazaki and T. Tsuchizaki (1987) On the infection of a BaYMV resistant cv. Misato Golden. *Ann. Phytopath. Soc. Jpn.* 53: 123.
- Okada, Y., S. Kashiwazaki, R. Kanatani, S. Arai and K. Ito (2003a) Effect of barley yellow mosaic disease resistant gene *rym1* on the infection by strains of Barley yellow mosaic virus and Barley mild mosaic virus. *Theor. Appl. Genet.* 106: 181-189.
- Okada, Y., R. Kanatani, S. Arai, T. Asakura and K. Ito (2003b) Production of a novel virus-resistant barley line introgression to the *rym1* locus with high malting quality using DNA marker assisted selection. *J. Inst. Brew.* 109: 99-102.
- Okada, Y., R. Kanatani, S. Arai and K. Ito (2003c) A CAPS marker that distinguishes the barley yellow mosaic disease resistance locus *rym1* derived from Chinese landrace 'Mokusekko 3'. *J. Inst. Brew.* 109: 103-105.
- Sambrook, J., E.F. Fritsch and T. Maniatis (1989) *Molecular Cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory, New York 9.31-9.62.
- Toyama, A. and T. Kusaba (1970) Transmission of soil-borne barley yellow mosaic virus. 2. *Polymyxa graminis* Led. As vector. *Ann. Phytopath. Soc. Jpn.* 36: 223-229.
- Ukai, Y. and A. Yamashita (1980) Induced mutation for resistance to barley yellow mosaic virus. *Jpn. J. Breed.* 30: 125-130 (in Japanese with English summary).