

# Mapping of quantitative trait loci for *Melon yellow spot virus* resistance in cucumber (*Cucumis sativus* L.)

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**Abstract** *Melon yellow spot virus* (MYSV), a member of the genus *Tospovirus*, is a devastating thrips-transmitted virus of cucurbits. The cucumber accession 27028930 was found to be resistant to MYSV in our previous study. In the present study, we identified quantitative trait loci (QTLs) conferring resistance to two MYSV isolates, MYSV-FuCu05P and MYSV-S. An F<sub>2</sub> population derived from a cross between accession 27028930 and the susceptible cultivar ‘Tokiwa’ was used to test for MYSV-FuCu05P resistance and to construct a genetic linkage map. The map contains 7 linkage groups and is comprised of 81 simple sequence repeat (SSR) markers, which span 715 cM, with an average interval between markers of 9.7 cM. Two major QTLs [on chromosomes (Chr.) 1 and 3] and one minor QTL (Chr. 7) contributed by 27028930, and one minor QTL (Chr. 4) contributed by ‘Tokiwa’ were detected for

MYSV-FuCu05P resistance. Another F<sub>2</sub> population from the same cross was used to test for MYSV-S resistance and to construct a genetic linkage map. The latter map also contains 7 linkage groups and is comprised of 76 SSR markers, which span 674 cM with an average interval between markers of 9.8 cM. A resistance gene for MYSV-S was identified on Chr. 3 by linkage analysis. Using independent populations, we demonstrated that SSR markers linked to the major QTLs for MYSV-FuCu05P resistance on Chr. 1 and 3 and an SSR marker linked to a resistance gene for MYSV-S on Chr. 3 could be used to select for MYSV resistance in cucumber breeding.

**Keywords** Framework map · MYSV · QTL · Spotted wilt · SSR marker · *Tospovirus*

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## Introduction

*Melon yellow spot virus* (MYSV), a member of the genus *Tospovirus*, is a devastating thrips-transmitted virus of cucurbits in Japan (Kato et al. 2000; Takeuchi et al. 2001). The areas affected by MYSV are increasing, and MYSV has also been reported in Taiwan, Thailand, China and Ecuador (Chen et al. 2008; Chiemsombat et al. 2008; Gu et al. 2012; Quito-Avila et al. 2014). MYSV induces chlorotic spots, mosaic mottling, leaf yellowing, and sometimes causes mottling on the fruits of cucumber (*Cucumis*

*sativus* L.). These symptoms cause yield loss and reduce fruit quality. MYSV is transmitted in a persistent manner by melon thrips (*Thrips palmi* Karny) and is not transmitted via soil contamination or seeds (Kato et al. 1999). Two MYSV isolates have been reported in Japan, MYSV-S (originally isolated from melon) and MYSV-FuCu05P (isolated from cucumber) (Kato et al. 1999; Sugiyama et al. 2009a; Takeuchi et al. 2001). The host range differs between the isolates: MYSV-S also infects watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai), squash (*Cucurbita maxima* L.), and bottle gourd (*Lagenaria siceraria* (Mol.) Stand.), whereas MYSV-FuCu05P only infects cucumber and hardly infects melon (Kato et al. 1999; Takeuchi et al. 2001). MYSV-S is not reported in Japan at present, but a similar isolate has been reported in Thailand (Wiboonchotikorn et al. 2012). The amino acid sequence of the nucleocapsid protein of the Thai isolate was identical to that of MYSV-S.

MYSV is currently managed by cultivation practices such as removing infected plants, and using reflective mulch, insect-proof netting, and chemical treatment against its vector (Okuda et al. 2009). One of the most effective methods of disease control is the use of resistant cultivars. However, no cucumber or melon cultivars have been reported to be resistant to MYSV. Sugiyama et al. (2009a) evaluated 398 cucumber accessions for resistance to MYSV-FuCu05P and MYSV-S, and found that accession 27028930 originating from Thailand was resistant to both isolates. Necrotic spots did not develop on MYSV-FuCu05P-inoculated cotyledons of 27028930 at 20 °C, whereas higher incubation temperatures (25 or 30 °C) facilitated the appearance of necrotic spots (Sugiyama et al. 2009b). Accession 27028930 was systemically infected by MYSV-FuCu05P at all temperatures tested, but symptoms were mild. No systemic infection by MYSV-S developed in 27028930 plants at 20 °C. Higher temperatures (25 or 30 °C) facilitated systemic infection by MYSV-S, but the percentage of infected plants was lower in comparison with a susceptible cultivar (Sugiyama et al. 2009b). These results show that accession 27028930 can be used as breeding material for MYSV resistance, although there is no genetic information on MYSV resistance of 27028930.

Recently, a number of SSR markers (Cavagnaro et al. 2010; Ren et al. 2009), a genome sequence (Cavagnaro et al. 2010; Huang et al. 2009; Wóycicki

et al. 2011), high-density genetic linkage maps (Fukino et al. 2013; Ren et al. 2009; Yang et al. 2013), and an integrated genetic map (Zhang et al. 2012) became available for cucumber. In addition, QTL analyses for cucumber resistance to diseases such as powdery mildew (*Podosphaera xanthii*), downy mildew [*Pseudoperonospora cubensis* (Berk. & Curt.) Rostov], and *Cucurbit yellow stunting disorder virus* (CYSDV) have been reported (de Ruiter et al. 2008; Fukino et al. 2013; He et al. 2013; Sakata et al. 2006; Yoshioka et al. 2014). But the number of DNA markers available for marker-assisted selection (MAS) in cucumber is still not enough. To develop DNA markers for MAS, it is first necessary to determine broad genomic regions controlling the traits of interest by QTL mapping, and then to narrow them by fine mapping. If we have a versatile and genome-wide marker set which can be used for different mapping populations, it will facilitate development of a framework map for mapping genes of interest, and of DNA markers for MAS.

The objectives of this study were to develop an versatile and genome-wide marker set for construction of a framework map, to identify QTLs conferring resistance to the MYSV isolates, MYSV-FuCu05P and MYSV-S, and to develop DNA markers linked to MYSV resistance.

## Materials and methods

### Plant materials

For selection of polymorphic SSR markers, 6 genotypes of cucumber (27028930, ‘Tokiwa’, ‘CS-PMR1’, ‘Santou’, ‘Kaga Aonaga-fushinari’, and an inbred of F<sub>1</sub> hybrid ‘Encore10’) were used. Five genotypes of cucumber (27028930, ‘Tokiwa’, ‘Kyuri Chukanbohon Nou 4’, ‘Kyuri Ano 4’, and ‘High Green 21’) were used as parental lines. The accession 27028930 is an MYSV-resistant accession originating from Thailand. ‘Tokiwa’ and ‘Kyuri Chukanbohon Nou 4’ are susceptible inbred cultivars. ‘High Green 21’ is a susceptible F<sub>1</sub> cultivar. ‘Kyuri Ano 4’ is an MYSV-resistant inbred line, which is the progeny of a cross of 27028930, ‘Tokiwa’, and ‘Encore 10’. These 5 genotypes were used to develop F<sub>1</sub> and F<sub>2</sub> populations.

Ninety-two F<sub>2</sub> plants (‘Tokiwa’ × 27028930) were used for framework map construction and QTL analysis

for resistance to MYSV-FuCu05P. Ninety-three F<sub>2</sub> plants ('Kyuri Chukanbohon Nou 4' × 'Kyuri Ano 4') were used to test for association between marker genotypes and MYSV-FuCu05P resistance. Ninety-one F<sub>2</sub> plants ('Tokiwa' × 27028930) were used for framework map construction and linkage analysis for resistance to MYSV-S. Ninety F<sub>2</sub> plants ('High Green 21' × 27028930) were used to test for association between marker genotypes and MYSV-S resistance.

#### Virus materials

MYSV-FuCu05P (originally isolated from cucumber; Sugiyama et al. 2009a) and MYSV-S (isolated from melon; Kato et al. 2000) were rub-inoculated as described below onto fully expanded cotyledons of cucumber cultivar 'Shimoshirazu'. Systemically infected leaves were stored at −80 °C and used to prepare the inocula.

#### MYSV resistance tests

For the MYSV-FuCu05P resistance test, seeds of F<sub>2</sub> ('Tokiwa' × 27028930), parental lines, and F<sub>1</sub> were sown on January 5, 2010. Inoculum was prepared by grinding infected cucumber leaves at a 1:10 ratio (w/v) in 100 mM phosphate buffer (pH 7.0) containing 0.1 % (v/v) 2-mercaptoethanol. Plants were inoculated 7 days after sowing by rubbing carborundum-dusted cotyledons with cotton pieces soaked in the inoculum. The inoculated plants were grown in a growth chamber (LH-300, Nippon Medical & Chemical Instruments, Osaka, Japan) at 20 °C under 14-h light/10-h dark conditions. Then the plants were planted in single-row plots, 0.4 m apart within rows and 1.2 m between rows, in a greenhouse on January 28, 2010. During the cultivation period, the maximum, minimum, and average air temperatures in the greenhouse were 37.0, 12.4, and 20.5 °C, respectively. All lateral branches were removed and the main vine of each plant was pinched at 1.8-m height. Because all plants were systemically infected, the resistance level of each plant to MYSV-FuCu05P was evaluated using the disease severity index (DI) recorded as follows: 0 = no symptoms, 1 = only mild vein yellowing, 2 = only slight mosaic mottling, no yellowing, 3 = mild yellowing, 4 = yellowing, 5 = severe yellowing, and 6 = dead (Fig. 1). DIs of the 5th to 14th leaves were recorded at 76 days post-inoculation (dpi); average DI calculated for each plant was used

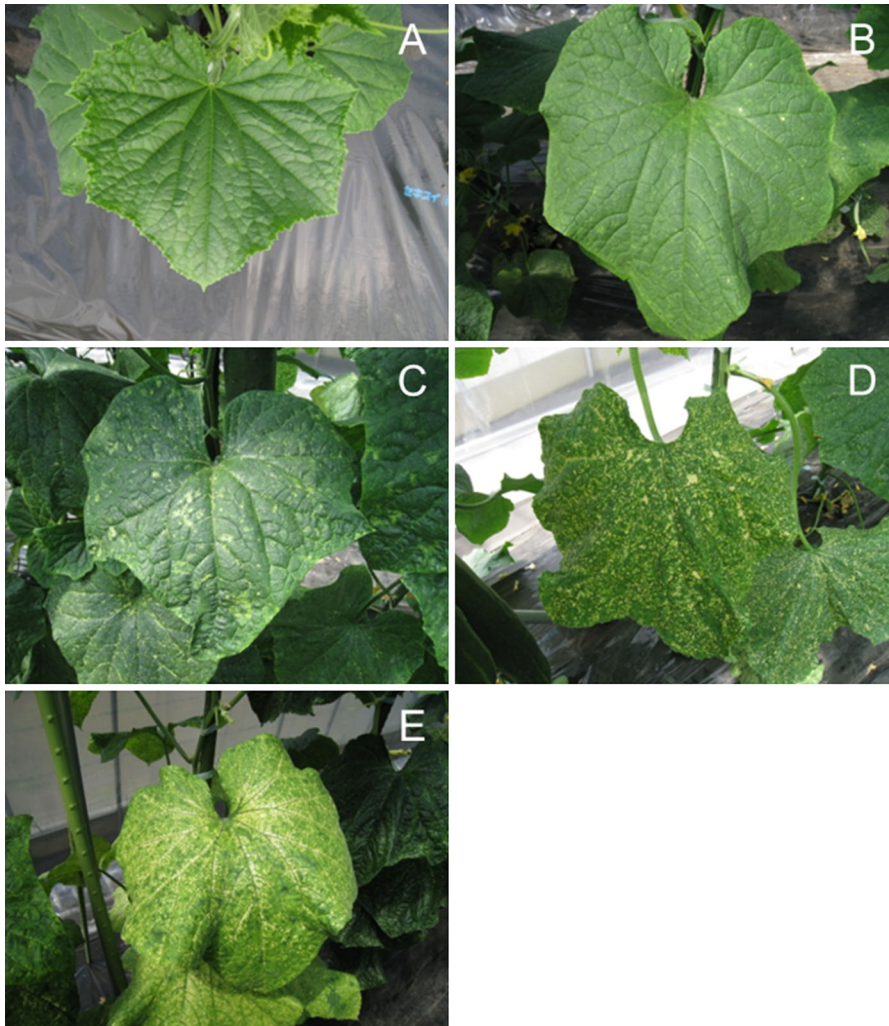
for QTL analysis. Seeds of F<sub>2</sub> ('Kyuri Chukanbohon Nou 4' × 'Kyuri Ano 4'), parental lines, and F<sub>1</sub> for the MYSV-FuCu05P resistance test were sown on September 25, 2012. The plants were planted in a green house on October 10, and DI was recorded at 64 dpi. Viral inoculation and plant cultivation were performed as described above.

For the MYSV-S resistance test, seeds of F<sub>2</sub> ('Tokiwa' × 27028930), parental lines, and F<sub>1</sub> were sown on February 15, 2010. MYSV-S was inoculated 7 days after sowing by the same method as that used for MYSV-FuCu05P. Plants were grown in a growth chamber as described above. The third true leaf of each plant was sampled at 28 dpi, and MYSV-S was detected by using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams 1977) as previously described (Sugiyama et al. 2009b). Plants with detectable virus were recorded as susceptible and those with no detectable virus were classed as resistant. Seeds of F<sub>2</sub> ('High Green 21' × 27028930), parental lines, and F<sub>1</sub> for the MYSV-S resistance test were sown on February 15, 2011. Viral inoculation and plant cultivation were performed as above.

#### SSR marker analysis

Genomic DNA was isolated from young true leaves of cucumber by using a DNeasy 96 Plant Kit (Qiagen, Hilden, Germany). SSR markers from previous studies (Ren et al. 2009; Fukino et al. 2008; Yuan et al. 2008; Watcharawongpaiboon and Chunwongse 2008) were used to select polymorphic DNA markers. PCR amplification was carried out in a 10-μL solution containing 1–5 ng of genomic DNA, 1 μM of each primer, and 5 μL of GoTaq Master Mix (Promega, Madison, WI, USA). Reaction conditions were as follows: 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min; and 72 °C for 5 min. Products were labelled by post-PCR labelling (Kukita and Hayashi 2002) by using R110-ddUTP (PerkinElmer, Waltham, MA, USA). The sizes of the amplified fragments were estimated by using an automated DNA analyzer (model 3730xl, Applied Biosystems, Foster City, CA, USA) with a GeneScan-500LIZ size standard (Applied Biosystems). Fragment length was determined with GeneMapper software (Applied Biosystems).

For construction of framework maps, forward primers were labelled with fluorescent dyes, 6-FAM, VIC, NED or PET (Applied Biosystems). PCR was



**Fig. 1** Leaves of cucumber (*Cucumis sativus* L.) plants inoculated with MYSV-FuCu05P. The disease severity indices (DI) were defined as follows: 0 no symptoms, 1 only mild vein

yellowing (a), 2 only slight mosaic mottling, no yellowing (b), 3 mild yellowing (c), 4 yellowing (d), 5 severe yellowing (e), and 6 = dead

carried out in an 8- $\mu$ L reaction mix containing 1–5 ng of genomic DNA, 4  $\mu$ L of GoTaq Master Mix, and 0.4  $\mu$ M of each primer. Reaction conditions were as follows: 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min; and 72 °C for 5 min. The sizes of the amplified fragments were estimated as described above.

#### Framework map construction and QTL analysis

F<sub>2</sub> populations and SSR markers selected as described above were used to construct framework maps with

MAPMAKER/EXP 3.0 (Lander et al. 1987). Marker data were assigned to linkage groups (LGs) by using a minimum logarithm of odds (LOD) likelihood score of 4.0. The Kosambi map function (Kosambi 1943) was used to calculate the genetic distance between markers. The previously reported assignment of LGs to chromosomes (Ren et al. 2009) was used. QTL analysis was performed using composite interval mapping with Windows QTL Cartographer v2.5 (Wang et al. 2012). Putative QTLs were estimated from the calculated LOD threshold score after 1000 permutation tests.





**Fig. 2** Plants of accession 27028930 (a) and ‘Tokiwa’ (b) at 65 days post-inoculation with MYSV-FuCu05P. Leaves of 27028930 plants show slight mosaic mottling, mild yellowing (arrows in a), and leaves of ‘Tokiwa’ plants show severe yellowing (arrows in b)

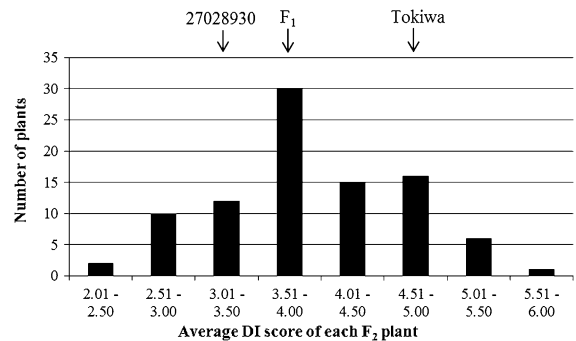
## Results

### Evaluation of resistance to MYSV-FuCu05P

All plants of  $F_2$  (‘Tokiwa’  $\times$  27028930), parental lines, and  $F_1$  showed mild mosaic mottling and yellowing of the 2nd and 3rd leaves at 14 dpi. Later, susceptible ‘Tokiwa’ plants showed severe leaf yellowing, whereas resistant 27028930 plants showed mild yellowing (Fig. 2). At 76 dpi, the DI scores of  $F_2$  plants (‘Tokiwa’  $\times$  27028930) ranged from 2.2 to 5.7 (Fig. 3). The mean DI score of 27028930 was  $3.3 \pm 0.18$  [standard deviation (SD)] and that of ‘Tokiwa’ was  $4.8 \pm 0.20$ . The mean DI score of  $F_1$  plants (‘Tokiwa’  $\times$  27028930) was  $3.9 \pm 0.15$ , which was between those of 27028930 and ‘Tokiwa’. The mean DI score of  $F_1$  plants (‘Tokiwa’  $\times$  27028930) was between those of 27028930 and ‘Tokiwa’ ( $3.9 \pm 0.15$ ), which indicates an incomplete-dominant phenotype.

### Evaluation of resistance to MYSV-S

MYSV-S was detected in all ‘Tokiwa’ plants, but not in any of the 27028930 plants (Table 1). MYSV-S was detected in 1 out of 10  $F_1$  plants (‘Tokiwa’  $\times$  27028930),



**Fig. 3** Frequency distribution of average disease index (DI) scores of  $F_2$  plants (‘Tokiwa’  $\times$  27028930) inoculated with MYSV-FuCu05P. DI scores were recorded as following: 0 no symptoms, 1 only mild vein yellowing, 2 only slight mosaic mottling, no yellowing, 3 mild yellowing, 4 yellowing, 5 severe yellowing, and 6 dead. The average DI score for leaves from 5th to 14th of each plant was calculated. The average DI scores for the parental cultivars (27028930 and ‘Tokiwa’) and the  $F_1$  generation are indicated above the graph

1 out of 10  $F_1$  plants (27028930  $\times$  ‘Tokiwa’), and 27 out of 91  $F_2$  plants (‘Tokiwa’  $\times$  27028930). These results suggest that resistance is controlled by a single dominant gene (*Sws*; resistance to spotted wilt by MYSV-S) in agreement with the expected segregation ratio ( $\chi^2 = 0.91$ ;  $P = 0.19$ ).

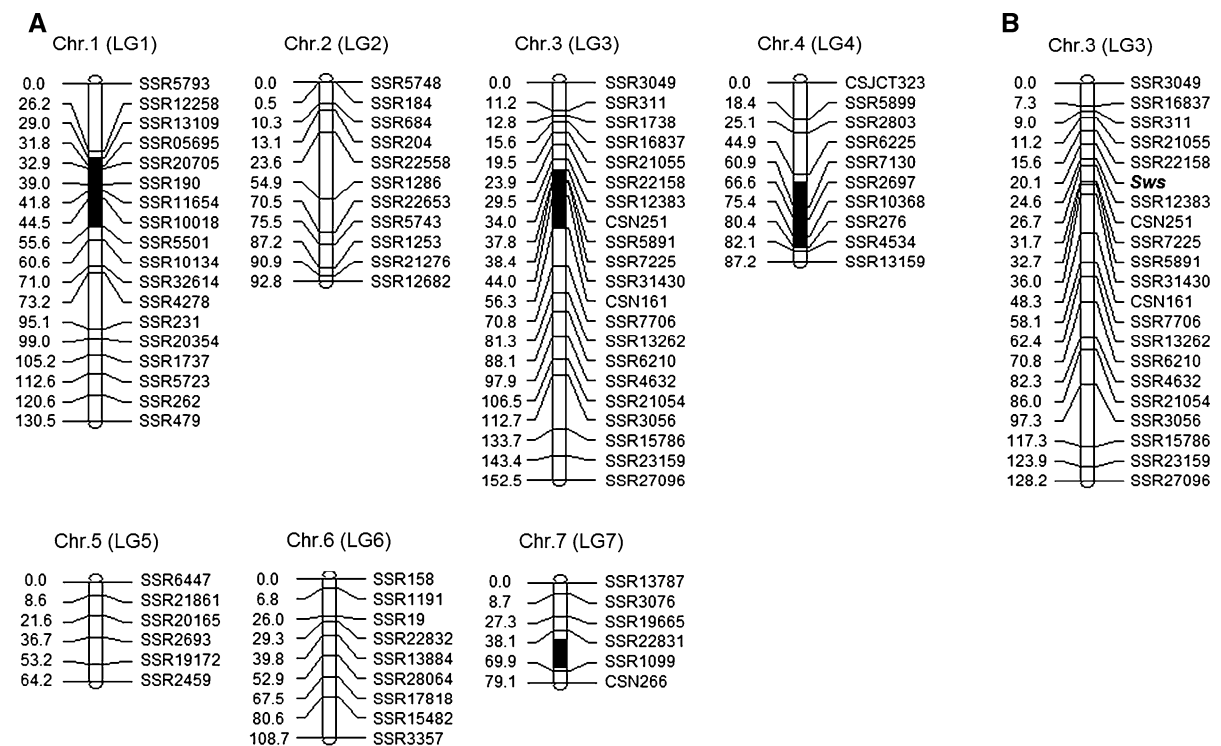
**Table 1** MYSV-S resistance of F<sub>1</sub> and F<sub>2</sub> plants derived from a cross between 27028930 and ‘Tokiva’

Population	Number of plants		
	Resistant	Susceptible	Total
27028930 (27)	10	0	10
Tokiwa(T)	0	10	10
F <sub>1</sub> (T × 27)	9	1	10
F <sub>1</sub> (27 × T)	9	1	10
F <sub>2</sub> (T × 27)	64	27	91

### Construction of framework maps

A total of 908 SSR markers, including 795 from Ren et al. (2009), 101 from Fukino et al. (2008), 11 from Yuan et al. (2008), and 1 from Watcharawongpaiboon and Chunwongse (2008), were used to detect polymorphisms among 27028930, ‘Tokiva’, ‘CS-PMR1’, ‘Santou’, ‘Kaga Aonaga-fushinari’, and ‘Encore10’.

Markers for framework map construction were selected by using the following criteria: (1) polymorphic between at least 2 pairs of 6 genotypes, (2) a map distance between neighboring markers of 10–30 cM as inferred from the linkage maps of Ren et al. (2009) and Fukino et al. (2013), and (3) marker polymorphisms are clear and reproducible. Framework maps were constructed by using the marker set and the F<sub>2</sub> populations (‘Tokiva’ × 27028930) used for the MYSV-FuCu05P and MYSV-S resistance tests. The map constructed by using the former population contained 7 LGs and comprised 81 SSR markers, which spanned 715 cM with an average interval between markers of 9.7 cM (Fig. 4a). The map constructed by using the latter population also contained 7 LGs and comprised 76 SSR markers, which spanned 674 cM with an average interval between markers of 9.8 cM. The order of the markers and the distance between them in each LG were similar in the two maps. The versatility of the marker set was



**Fig. 4** Cucumber (*Cucumis sativus* L.) linkage maps constructed by using F<sub>2</sub> populations (‘Tokiva’ × 27028930). Linkage group (LG) and chromosome (Chr.) numbers are shown. **a** A linkage map constructed by using the F<sub>2</sub> population for the MYSV-FuCu05P resistance test. Black blocks indicate

significant QTLs (*Swf-1* on LG1, *Swf-2* on LG3, *Swf-3* on LG4, and *Swf-4* on LG7) with LOD > 3.4. **b** A linkage map of LG3 constructed by using the F<sub>2</sub> population for the MYSV-S resistance test

**Table 2** QTLs for MYSV-FuCu05P resistance in F<sub>2</sub> plants ('Tokiwa' × 27028930)

QTL	Linkage group	Flanking markers	Position (cM)	Maximum LOD	Additive effect <sup>a</sup>	R <sup>2</sup> (%)
<i>Swf-1</i>	1	SSR13109–SSR5501	29.0–55.6	10.5	−0.47	20.1
<i>Swf-2</i>	3	CSN251–CSN161	34.0–56.0	11.0	−0.50	22.1
<i>Swf-3</i>	4	SSR6225–SSR276	61.9–80.4	4.4	0.40	13.8
<i>Swf-4</i>	7	SSR22831–SSR1009	50.1–68.1	4.9	−0.38	9.4

<sup>a</sup> Additive main effect of the parent contributing the higher-value allele: positive and negative values indicate that higher-value alleles come from 'Tokiwa' and 27028930, respectively

confirmed by using two additional F<sub>2</sub> populations (CS-PMR1 × 'Santou' and CS-PMR1 × 'Kaga Aonaga-fushinari'; data not shown).

#### QTL analysis for resistance to MYSV- FuCu05P and linkage analysis for resistance to MYSV-S

Two major QTLs (*Swf-1* [resistance to spotted wilt caused by MYSV-FuCu05P-1] on Chr. 1 and *Swf-2* on Chr. 3) and one minor QTL (*Swf-4* on Chr. 7) contributed by 27028930, and one minor QTL (*Swf-3* on Chr. 4) contributed by 'Tokiwa' were detected for MYSV-FuCu05P resistance (Fig. 4a). The major QTL on Chr. 1 explained 20.1 % of the phenotypic variance with a maximum LOD of 10.5, whereas the major QTL on Chr. 3 explained 22.1 % of the phenotypic variance with a maximum LOD of 11.0 (Table 2). In a linkage analysis for MYSV-S resistance, a resistance gene (*Sws*) was mapped on Chr. 3 between SSR22158 and SSR 12383 at a distance of 4.5 cM from both markers (Fig. 4b).

#### Association between marker genotypes and resistance

To assess whether the SSR markers linked to the QTLs and *Sws* could be used to select for MYSV resistance, we tested their association with resistance in F<sub>2</sub> populations derived from a cross between different parental lines. SSR markers linked to the QTLs on Chr. 1 and Chr. 3 and F<sub>2</sub> population ('Kyuri Chukanbohon Nou 4' × 'Kyuri Ano 4') were used to examine the relationship between the markers and MYSV-FuCu05P resistance (Table 3). The mean DI score of F<sub>2</sub> plants homozygous for both alleles derived from the resistant line 'Kyuri Ano 4' was 2.6 ± 0.8 (genotype A/A) at 64 dpi. The mean DI score of F<sub>2</sub> plants homozygous for both alleles derived from the

susceptible cultivar 'Kyuri Chukanbohon Nou 4' was 4.8 ± 0.4 (genotype B/B). The mean DI scores of genotypes A/B and B/A were 3.8 ± 0.5 and 3.0 ± 0.6, respectively. The DI score of genotype A/A was significantly different from those of genotypes A/B and B/B at the 5 % level. These results show that the SSR markers linked to the major QTLs can be used to select for resistance to MYSV-FuCu05P.

An SSR marker linked to *Sws* on Chr. 3 and F<sub>2</sub> population ('High Green 21' × 27028930) was used to examine the relationship between the marker genotype and MYSV-S resistance (Table 4). MYSV-S was detected in 21 of 22 plants homozygous for the allele derived from the susceptible cultivar 'High Green 21' but only in 5 of 28 plants homozygous for the allele derived from the resistant line 27028930 and only in 9 of 40 plants heterozygous for the allele. These results show that the SSR marker linked to *Sws* on Chr. 3 can be used to select for resistance to MYSV-S.

#### Discussion

In the present study, we developed a genome-wide SSR marker set by using 6 cucumber genotypes, which could be used for construction of framework maps for mapping populations of interest. Then we constructed framework maps by using the F<sub>2</sub> populations used for the MYSV resistance tests, and performed QTL analysis. This is the first report of QTL analysis for resistance to MYSV in cucumber. Two major and two minor QTLs were detected for MYSV-FuCu05P resistance (Fig. 4a), and DNA markers linked to the major QTLs were shown to be effective for selection of MYSV-FuCu05P-resistant plants (Table 3). The F<sub>2</sub> plants homozygous for both alleles on Chr. 1 and Chr.

**Table 3** Relationship between genotypes of markers SSR20705 and SSR31430 and average disease index (DI) scores of F<sub>2</sub> plants derived from a cross between resistant Kyuri Ano 4 and susceptible ‘Kyuri Chukanbohon Nou 4’

Population	Marker genotypes <sup>a</sup>		Number of plants tested	Average DI scores <sup>b</sup>
	SSR20705 (LG1)	SSR31430 (LG3)		
Kyuri Ano 4 (A)	A	A	10	1.6 ± 0.1
Kyuri Chukanbohon Nou 4 (N)	B	B	10	5.8 ± 0.2
F <sub>1</sub> (N × A)	H	H	10	3.3 ± 0.2
F <sub>2</sub> (N × A)	A	A	4	2.6 ± 0.8 a
	A	B	7	3.8 ± 0.5 bcd
	A	H	11	3.5 ± 0.5 abc
	B	A	9	3.0 ± 0.6 ab
	B	B	3	4.8 ± 0.4 de
	B	H	13	3.9 ± 0.7 cd
	H	A	13	3.1 ± 0.6 ab
	H	B	10	4.8 ± 0.7 e
	H	H	23	4.1 ± 0.6 cde

<sup>a</sup> Marker genotype designation: A, homozygous for 27028930; H, heterozygous; B, homozygous for ‘Kyuri Chukanbohon Nou 4’ allele

<sup>b</sup> Values represent mean ± SD. Values followed by the same letter are not significantly different at the 5 % level, as determined by the Tukey–Kramer HSD test

**Table 4** Relationship between genotypes of the marker SSR12383 and MYSV-S resistance of F<sub>2</sub> plants derived from a cross between resistant 27028930 and susceptible ‘High Green 21’

Population	Marker genotype <sup>a</sup>	Number of plants tested	Number of plants with MYSV-S detection	Percentage of plants with MYSV-S detection
27028930 (27)	A	8	0	0.0
High Green 21 (H)	B	8	8	100.0
F <sub>1</sub> (H × 27)	H	8	0	0.0
F <sub>2</sub> (H × 27)	A	28	5	17.9
	B	22	21	95.5
	H	40	9	22.5

<sup>a</sup> Marker genotype designation: A homozygous for 27028930; H heterozygous, B homozygous for ‘High Green 21’ allele

3 (genotype A/A) derived from the MYSV-resistant inbred line ‘Kyuri Ano 4’ showed the lowest DI. To develop an F<sub>1</sub> hybrid cucumber cultivar resistant to MYSV-FuCu05P, it is better to chose parental lines that both have genotype A/A because the resistance of plants with genotype H/H was lower than that of plants with genotype A/A (Table 3). Although the resistant line ‘Kyuri Ano 4’ was systemically infected with MYSV-FuCu05P, accumulation of the virus in true leaves of ‘Kyuri Ano 4’ was lower than in the leaves of susceptible cultivars (data not shown). Thus, the QTLs for MYSV-FuCu05P resistance partially inhibit systemic infection by the virus, although the mechanism is unclear.

MYSV is a member of the genus *Tospovirus*, and the dominant MYSV-S resistance gene *Sw5* was mapped between SSR22158 and SSR12383 on Chr. 3 (Fig. 4b). Many dominant virus resistance genes have been isolated (Maule et al. 2007), and most of them encode proteins with nucleotide binding site–leucine rich repeat (NBS-LRR) domains. Several *tospovirus* resistance genes have been reported in tomato (*Solanum lycopersicum* L.). The tomato *Sw5* gene conferring resistance to *Tomato spotted wilt virus* (TSWV) has been identified (Spasova et al. 2001). *Sw5* belongs to the coiled coil (CC)-NBS-LRR group of plant resistance genes, and its locus contains at least six paralogues (Folkertsma et al. 1999, Rehman et al.



2009). Among these paralogues, *Sw5-b* gene is necessary and sufficient for conferring resistance to TSWV, as demonstrated in *Sw5-b* transformed *Nicotiana tabacum* plants (Spasova et al. 2001). The genome of the cucumber line Gy14 contains 70 NBS-LRR type R gene homologs (Yang et al. 2013). One of these genes (Cucs041670) has been mapped close to the marker SSR22158 flanking *Sws* on Chr. 3, and *Swf-2* was also detected near *Sws*; further studies are needed to determine the relationship among Cucs041670, *Sws*, and *Swf-2*. In 27028930 plants inoculated with MYSV-S, the virus was detected but its spread was suppressed in inoculated leaves, and no systemic infection developed at 20 °C (Sugiyama et al. 2009b). Therefore, the resistance of 27028930 to MYSV-S involves restriction of long-distance movement of the virus. Plant viruses move over short distances (from cell to cell) via plasmodesmata and over long distances via the vascular system, and virus movement requires compatibility between virus-encoded movement factors and host components (Carrington et al. 1996). In *A. thaliana*, *RTM1* (restricted TEV movement 1) and *RTM2* are expressed in phloem-associated tissues and restrict the long-distance movement of *Tobacco etch virus* (TEV) (Chisholm et al. 2001). Caranta et al. (2002) reported that one major QTL and several minor QTLs were involved in a partial restriction of the long-distance movement of *Cucumber mosaic virus* (CMV) in pepper (*Capsicum annuum* L.). *Sws* also restricts the long-distance movement of MYSV-S, but further studies are needed to ascertain the mechanism of MYSV-S resistance.

MYSV is transmitted by thrips in a field, while plants in this study were inoculated mechanically. Distinct responses of pepper (*Capsicum* spp.) accessions after mechanical and thrips-mediated inoculation with TSWV have been reported (Boiteux et al. 1993): some accessions which showed resistance to TSWV in a field were highly infected with TSWV after mechanical inoculation. Preliminary results on MYSV inoculation showed that the percentage of infected 27028930 plants was lower than that of susceptible cultivars after thrips-mediated inoculation with MYSV-S, and that the symptoms on upper leaves of 27028930 were milder than those of susceptible cultivars after thrips-mediated inoculation with MYSV-FuCu05P (data not shown). Therefore, plants which show resistance to MYSV by mechanical

inoculation are expected to be resistant to MYSV under natural field conditions.

In cucumber, genetic mapping of resistance genes to CYSDV, *Papaya ringspot virus* (PRSV), and *Zucchini yellow mosaic virus* (ZYMV) has been reported (de Ruiter et al. 2008; Park et al. 2000, 2004), but there have been no reports of cloning of cucumber disease resistance genes. Recently, Amano et al. (2013) identified a candidate gene for ZYMV resistance as the vacuolar protein sorting-associated protein 4-like gene. The authors found that 2 SNPs in this gene were associated with the ZYMV resistance phenotype. Based on these SNPs, they developed DNA markers that can be used for MAS for ZYMV resistance in cucumber breeding. In this study, we demonstrated that SSR markers linked to the QTLs for MYSV-FuCu05P resistance or linked to *Sws* are effective for selection of MYSV-resistant plants. Our results will accelerate fine mapping and identification of MYSV resistance genes and development of MYSV-resistant cultivars by using MAS.

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