A New Tobamovirus – resistance Gene, L^{1a} , of Sweet Pepper (*Capsicum annuum* L.)

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Summary

Tobamoviruses are classified into four pathotypes, P_0 , P_1 , $P_{1,2}$ and $P_{1,2,3}$, based on the infection patterns in *Capsicum* plants that carry different L genes, L^1 – L^4 . *Paprika mild mottle virus* Japanese strain (PaMMV–J), a P_1 pathotype of *Tobamovirus*, systemically infected L^1 –homozygote *Capsicum* plants and induced mosaic on upper uninoculated leaves. The same virus induced systemic necrosis in 19 commercial cultivars and three breeding fixed lines of *Capsicum* plants resistant and susceptible to the P_0 and $P_{1,2}$ pathotypes of *Tobamovirus*, respectively. Genetic analysis showed that resistance to the P_0 pathotype of *Tobamovirus* of these cultivars and fixed lines differed from L^1 and depended on a locus, which may be located on or near the L gene locus. Therefore, we designate the new gene as L^{1a} . Both L^{1a} –homozygote and –heterozygote plants showed resistance to the P_0 pathotype of *Tobamovirus* when incubated at 24°C to 30°C, suggesting that resistance of the L^{1a} to P_0 pathotype of *Tobamovirus* is temperature– and gene dosage–independent. Interestingly, L^{1a} –homozygote but not –heterozygote plants are resistant to PaMMV–J when incubated at 24°C but not at 26°C, indicating that L^{1a} plants resistant to PaMMV–J are temperature– and gene dosage–dependent.

Key Words: *Capsicum annuum*, L^{1a} , *Paprika mild mottle virus*, tobamovirus.

Introduction

Tobamovirus is one of the most devastating virus pathogens of plants, including the pepper plant (*Capsicum* spp.). To control tobamovirus diseases, the resistant L genes have been incorporated into pepper plants. The L gene–mediated virus resistance is accompanied by the induction of hypersensitive response (HR), characterized by rapid cell death, which restricts the virus from spreading within and around the necrotic lesions. This resistance is conferred by four allelic genes at the locus L , L^1 – L^4 , which provide increased protection against different kinds of *Tobamovirus* (Boukema, 1980, 1982). Tobamoviruses that infect *Capsicum* plants are classified into four pathotypes, P_0 , P_1 , $P_{1,2}$, and $P_{1,2,3}$, based on the infection patterns in *Capsicum* plants that carry different L genes (Rast, 1988). Five species of *Tobamovirus*, including *Tobacco mosaic virus* (TMV, P_0), *Tomato mosaic virus* (ToMV, P_0), *Tobacco mild green mosaic virus* (TMGMV, P_0), *Paprika mild mottle virus* (PaMMV, P_1), and *Pepper mild mottle virus*

(PMMoV, $P_{1,2}$ or $P_{1,2,3}$) have been reported as pathogens of *C. annuum* so far in Japan (Hamada et al., 2003; Morishima et al., 2003; Nagai, 1981; Nagai et al., 1981; Tsuda et al., 1998).

Many commercial pepper cultivars that are resistant and susceptible to P_0 and $P_{1,2}$ pathotypes of *Tobamovirus*, respectively, have been cultivated in Japan (Nagai et al., 1981). The P_1 pathotype of *Tobamovirus*, PaMMV, has only been isolated in Europe and China (Sakata et al., 1997; Tobias et al., 1982), and its genetic information remains unclear. Therefore, it is not known whether L^1 or L^2 genes are incorporated into commercial cultivars of pepper plants cultivated and used as breeding sources in Japan. We previously reported that PaMMV–J was isolated from sweet pepper (*C. annuum* L.) cultivated in a greenhouse in Kochi Prefecture, Japan, and its genetic information was elucidated (Hamada et al., 2003). In this study, to discriminate L^1 from L^2 genes incorporated in 27 commercial cultivars and three lines used parents in breeding programs of pepper plants were examined. These test plants, resistant and susceptible to P_0 and $P_{1,2}$ pathotypes of *Tobamovirus*, respectively, were inoculated with PaMMV–J and their response symptoms recorded. Results in this study indicate that eight cultivars have L^1 gene, whereas 19 cultivars and three breeding fixed lines have a new L gene that differs from L^1 and L^2 .

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Materials and Methods

Inoculation with tobamoviruses

Virions of TMGMV-J (P_0 pathotype of *Tobamovirus*), PaMMV-J (P_1 pathotype of *Tobamovirus*) or PMMoV-J ($P_{1,2}$ pathotype of *Tobamovirus*) were purified from *N. benthamiana* inoculated with RNA transcripts of pPAJ (Hamada et al., 2003), pTGK16 (Morishima et al., 2003) or pTPW1 (Kirita et al., 1997), respectively. Leaves of 4-week-old seedlings from 27 varieties and three breeding fixed lines of *Capsicum* plants (Table 1) were inoculated with virions (10 μ g/ml). These test plants are resistant and susceptible to P_0 and $P_{1,2}$ pathotypes of *Tobamovirus*, respectively, as described by Hamada et al. (2003). Test plants were grown in 50 cell trays containing commercial soil (Tsuchitaro, Sumirin-Agri-Industrial, Aichi) in a growth chamber at 26°C under 10000 lux for 16 hr per day, and watered with five-times diluted Hoagland's solution (Hikichi et al., 1999). Detection of the virus on the inoculated leaves and upper uninoculated leaves at 7 and 14 days past inoculation (dpi), was performed by dot-immunobinding assay (DIBA, Suzuki et al., 1990). 'Shishitou chuukei' (*C. annuum*, L^+/L^+), 'Verbeterde Glas' (*C. annuum*, L^1/L^1), and 'Tabasco' (*C. frutescens*, L^2/L^2) that possess the L^+ , L^1 , and L^2 genes, respectively, were used as control cultivars.

Genetic analysis

To determine whether a fixed line, 'MK18-3-1' (*C. annuum*) has a new L gene, that differs from the L^1 gene, it was crossed with 'Verbeterde Glas' to produce F_1 plants. BC_1 plants were then produced by back-crossing 'MK18-3-1' or 'Verbeterde Glas' to the F_1 plants. F_2 plants were produced by selfing the F_1 progenies. Segregation ratios based on symptoms in F_1 , F_2 or BC_1 after inoculation with PaMMV-J were estimated.

Effect of temperature on L^{1a} -mediated resistance to P_0 pathotype of *Tobamovirus*

The efficacy of L^2 - L^4 genes depends on temperature (Boukema, 1980, 1982). To determine whether the efficacy of the new L gene, L^{1a} , was temperature-dependent, 'MK18-3-1', L^{1a} -heterozygote plants (L^{1a}/L^1 , MK18-3-1 $\times$ Verbeterde Glas), 'Shishitou chuukei', 'Verbeterde Glas', and 'Tabasco' were incubated at 24 or 30°C in the growth chamber with three plants after inoculation with TMGMV-J.

Effect of temperature on L^{1a} -mediated resistance to P_1 pathotype of *Tobamovirus*

To determine dependency of L^{1a} -mediated resistance to P_1 pathotype of *Tobamovirus* on temperature and gene dosage, three plants each of 'MK18-3-1', a L^{1a} -heterozygote cultivar, 'Tosa-hime' (*C. annuum*, L^{1a}/L^+), of which one parent was 'MK18-3-1', and 'Verbeterde Glas', were incubated at 24, 26, or 28°C after being

Table 1. Symptoms induced by PaMMV-J(P_1) on *Capsicum* plants resistant to P_0 pathotype and susceptible to $P_{1,2}$ pathotype of *Tobamovirus* and detection of the virus in the plants by DIBA.

Varieties	Symptoms/detection of virus ^z	
	Inoculated leaves	Upper uninoculated leaves
Verbeterde Glas ^y	SL/+	M/+
California Wonder	SL/+	M/+
Kyoh-midori	SL/+	M/+
Jumbo Red Pepper	SL/+	M/+
Hijiri	SL/+	M/+
Matsunomai	SL/+	M/+
Y-Pihman	SL/+	M/+
Poivron Esteral Hybrid F1	SL/+	M/+
Blondy	SL/+	M/+
Tosa-hime	VN/+	SN/+
Shishi-homare chuukei	VN/+	SN/+
Shishi-homare choukei	VN/+	SN/+
Kyoh-yutaka	VN/+	SN/+
Kyoh-nami	VN/+	SN/+
Beru-homare	VN/+	SN/+
Amejisuto	VN/+	SN/+
Orijinaru	VN/+	SN/+
Tosa-hikari D	VN/+	SN/+
Kiri-shima	VN/+	SN/+
Kanan	VN/+	SN/+
Kagayaki	VN/+	SN/+
Kagayaki K	VN/+	SN/+
Shiki-yutaka	VN/+	SN/+
Ryokuhou R	VN/+	SN/+
PGK2-8	VN/+	SN/+
Shin-sakigake 2	VN/+	SN/+
Nishi-midori	VN/+	SN/+
Tsubaki-gurihn	VN/+	SN/+
MK18-3-1 ^x	VN/+	SN/+
TMVR4-1 ^x	VN/+	SN/+
Kouen-tan1 ^x	VN/+	SN/+
Tabasco ^w	L/+	SL/-

^z SL, symptomless; VN, vein necrosis; L, local infection; M, mosaic; SN, systemic necrosis; +, detected; -, not detected.

^y L^1/L^1 check variety.

^x breeding fixed line.

^w L^2/L^2 check variety.

inoculated with PaMMV-J.

Results and Discussion

Infectivity of PaMMV-J to commercial cultivars of pepper plants and breeding fixed lines of *Capsicum* plants

When all test plants were inoculated with both TMGMV-J and PMMoV-J, necrotic lesions accompanied by HR and mosaic were induced on inoculated leaves and upper uninoculated leaves, respectively (data not shown). Movement of PaMMV-J was restricted in the inoculated leaves of 'Tabasco' (Table 1). On the other hand, inoculation with PaMMV-J resulted in detection of virions in inoculated and upper uninoculated leaves of the other tested plants at 7 and 14 dpi, respectively (Table 1), indicating that these plants possess L^1 -type resistance to *Tobamovirus*.

However, symptoms induced by PaMMV-J were divided into two phenotypes among the tested plants. First, no symptoms and mosaic appeared on inoculated leaves and upper uninoculated leaves of eight varieties (L^1 -type), respectively, similar to those observed on 'Verbeterde Glas' (L^1/L^1 , Table 1), suggesting that these

cultivars possess the L^1 gene. Second, vein necrosis and systemic necrosis became evident on inoculated and upper uninoculated leaves, respectively, on 19 other commercial cultivars and three breeding fixed lines containing a fixed line, 'MK18-3-1'. Hence, these plants may have a new resistant gene different from L^1 and L^2 .

Genetic analysis

Inoculation of all F_1 plants with PaMMV-J induced vein necrosis and systemic necrosis on inoculated and upper uninoculated leaves, respectively, similar to those observed on 'MK18-3-1' (Table 2). Symptoms on upper uninoculated leaves of F_2 plants produced by self-crossing of each F_1 plant segregated into two phenotypes, systemic necrosis and mosaic. The segregation ratio of systemic necrosis to mosaic was about 3:1; the goodness of fit was 0.8. Moreover, symptoms on upper uninoculated leaves of BC_1 plants, produced by back-crossing 'Verbeterde Glas' to the F_1 plants, also segregated into two phenotypes, systemic necrosis and mosaic at a ratio of about 1:1. On upper uninoculated leaves of BC_1 plants, only a systemic necrosis occurred (Table 2). Moreover, when test plants were inoculated

Table 2. Genetic analysis of resistance to PaMMV-J (P_1) based on symptoms on *Capsicum* plants.

Parents and Progeny	Symptoms on upper uninoculated leaves ^z		Total	Expected ratio ^y	χ^2 -value	Probability
	SN	M				
MK18-3-1	3 ^x	0	3	1:0	0.00	1.0
Verbeterde Glas	0	3	3	0:1	0.00	1.0
F_1 (MK18-3-1 × Verbeterde Glas)	6	0	6	1:0	0.00	1.0
F_2 (MK18-3-1 × Verbeterde Glas)	128	40	168	3:1	0.07	0.8
BC_1 ((MK18-3-1 × Verbeterde Glas) × Verbeterde Glas)	60	53	113	1:1	0.43	0.5
BC_1 ((MK18-3-1 × Verbeterde Glas) × MK18-3-1)	59	0	59	1:0	0.00	1.0

^z SN, systemic necrosis; M, mosaic.

^y expected segregation ratio in genetic analysis on the tobamovirus-resistant gene of MK18-3-1 independent of the L^1 gene.

^x Numbers of plants.

Table 3. Symptoms induced by TMGMV-J and detection of the virus by DIBA on L^{1a} -homozygote and heterozygote plants incubated at 24 and 30°C.

Varieties or F_1 progeny	Genotype	Symptoms/detection of virus ^z			
		24°C		30°C	
		I ^y	U	I	U
Shishitou chuukei	L^+/L^+	SL/+	M/+	SL/+	M/+
Verbeterde Glas	L^1/L^1	L/+	SL/-	SL/+	M/+
F_1 (MK18-3-1 × Verbeterde Glas)	L^{1a}/L^1	L/+	SL/-	L/+	SL/-
MK18-3-1	L^{1a}/L^{1a}	L/+	SL/-	L/+	SL/-
Tabasco	L^2/L^2	L/+	SL/-	VN/+	SN/+

^z SL, symptomless; L, local infection; M, mosaic; VN, vein necrosis; SN, systemic necrosis; +, detected; -, not detected.

^y I, inoculated leaves; U, upper uninoculated leaves.

with TMGMV-J, necrotic lesions accompanied by HR were induced on inoculated leaves of all plants (data not shown). These results indicate that resistance of 'MK 18-3-1' to P_0 pathotype of *Tobamovirus* depends on a gene, which is independent of and dominant to L^1 , and the gene segregates among offspring according to Mendel's laws. This gene may exist on or near the L locus. Therefore, we designated the gene as L^{1a} .

Effect of temperature on L^{1a} -mediated resistance to P_0 pathotype of Tobamovirus

TMGMV-J induced necrotic local lesions accompanied with HR on inoculated leaves of 'Verbeterde Glas' incubated at 24°C after inoculation. The infection of the virus was restricted in inoculated leaves (Table 3). On the other hand, when 'Verbeterde Glas' was incubated at 30°C after inoculation with TMGMV-J, no

symptom and mosaic were observed on the inoculated and upper uninoculated leaves, respectively. Although necrotic local lesions were induced on TMGMV-J-inoculated leaves of 'Tabasco' incubated at 24°C, necrosis was also induced on the upper uninoculated leaves incubated at 30°C (Table 3). Moreover, DIBA also showed that the virus moved systemically in both 'Verbeterde Glas' and 'Tabasco' incubated at 30°C. These results suggest that L^1 - and L^2 -mediated resistance to P_0 pathotype of *Tobamovirus* is overcome at 30°C.

However, when 'MK18-3-1' (L^{1a}/L^{1a}) and L^{1a}/L^1 plants (MK18-3-1 × Verbeterde Glas) inoculated with TMGMV-J were incubated at 24 or 30°C, necrotic local lesions appeared (Table 3). The HR was restricted to the inoculated leaves, indicating that 1) resistance due to the L^{1a} gene to P_0 pathotype of *Tobamovirus* is stable at high temperatures, unlike that found with the L^1 and L^2 genes and 2) resistance of L^{1a} to P_0 pathotype of *Tobamovirus* is dominant to that of L^1 and is not dependent on gene dosage.

Effect of temperature on L^{1a} -mediated resistance to P_1 pathotype of Tobamovirus

When L^1/L^1 plants inoculated with PaMMV-J were incubated at any of the temperatures in this study, no symptoms and mosaic were observed (Fig. 1A), but the virus was restricted in the inoculated leaves of L^2/L^2 plants incubated at 24, 26, and 28°C (data not shown). When inoculated L^{1a}/L^{1a} plants were incubated at 24°C, vein necrosis was induced on the inoculated leaves and did not become systemic (Fig. 1B), but at 26 or 28°C the infection became systemic. These results indicate that resistance of L^{1a}/L^{1a} plants to PaMMV-J is overcome at and above 26°C, so that L^{1a} -mediated resistance to PaMMV-J is temperature-dependent.

When, inoculated 'Tosa-hime' (L^{1a}/L^+) were incubated at 24 or 26°C, systemic necrosis was induced and the plants died (Fig. 1C), whereas at 28°C, mosaic were observed. DIBA showed that virus systemically moved in the L^{1a} heterozygote plants incubated at any temperature. These results suggest that L^{1a} -mediated resistance to PaMMV-J is gene dosage-dependent.

A coat protein is required for the elicitation of the L^2 - and L^3 -mediated resistance of *Capsicum* plants against the tobamoviruses (Berzal-Herranz et al., 1995; de la Cruz et al., 1997). Therefore, the resistance mediated by the L genes results from interactions between the products of the L genes and that of a corresponding avirulence gene, the coat protein gene of tobamoviruses. Our preliminary study suggested that elicitation of L^{1a} -mediated resistance to the P_0 pathotype of *Tobamovirus* also required a coat protein of the P_0 pathotype of *Tobamovirus* (Matsumoto, unpublished). Durable resistance of L^{1a}/L^{1a} plants to P_0 and P_1 pathotypes of *Tobamovirus* suggests that L^{1a}/L^{1a} plants may recognize both coat proteins of P_0 and P_1 pathotypes of

Fig. 1. Symptoms on 'Verbeterde Glas' (L^1/L^1 , A), 'MK18-3-1' (L^{1a}/L^{1a} , B) and 'Tosa-hime' (L^{1a}/L^+ , C) inoculated with PaMMV-J (P_1) and incubated at 24, 26, or 28°C.

Tobamovirus, although amino acid sequence identity of the coat protein of TMGMV-J and ToMV with PaMMV-J is 58.4% and 49.6%, respectively (Hamada et al., 2003; Morishima et al., 2003; Ohno et al., 1984).

We recommend that sweet peppers in greenhouse be cultivated at 26 to 30°C because our results demonstrate that L^{1a} -heterozygote plants cultivated at elevated temperatures are resistance to P_0 pathotype of *Tobamovirus*, whereas L^1 -mediated and L^2 -mediated resistances to P_0 pathotype of *Tobamovirus* are temperature-dependent. Moreover, it is difficult to produce progeny by crossing 'Tabasco' (L^2/L^2) to *C. annuum*. Therefore, L^{1a} -heterozygote pepper plants are the answer to control *Tobamovirus* disease in Japan prior to the introduction of $P_{1,2}$ pathotype.

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ピーマンが有する新しいトバモウイルス抵抗性遺伝子 L^{la} 澤田博正^{1,2}・竹内繁治¹・浜田博幸^{2*}・木場章範²・松本満夫¹・曳地康史²¹ 高知県農業技術センター 783-0023 高知県南国市² 高知大学農学部 783-8502 高知県南国市

摘 要

Capsicum 属植物が有する抵抗性遺伝子 L (L^1 - L^4) とトバモウイルスの病原型は階層的な関係を示すが、日本ではこれまで P_1 型トバモウイルスが未検出であったため、 L^1 と L^2 の分類を行うことができなかった。本研究では、 L^1 または L^2 を有するピーマン品種の分別を目的として、 P_1 型トバモウイルス *Paprika mild mottle virus* Japanese strain (PaMMV-J) をピーマン 30 品種・系統へ接種し、病徴と DIBA 法によるウイルス感染について調査した。8 品種で、 L^1 を有する *C. annuum* cv. Verbeterde Glas と同様に、上位葉にモザイクが誘導された。一方、22 品種・系統のピーマンには、PaMMV-J は全身感染し、全身で壊死が認められ

た。*C. frutescens* cv. Tabasco と同様に、接種葉に過敏感反応が誘導された品種は認められなかった。全身に壊死を呈した固定系統 'MK18-3-1' と L^1/L^1 植物との F_1 , F_2 および BC1 世代に PaMMV-J を接種した検定結果から、全身の壊死症状には L^1 とは異なる遺伝子が関与することが示唆され、この遺伝子を L^{la} と呼ぶことを提唱した。PaMMV-J は、26°C で栽培した L^{la}/L^{la} 植物に全身感染したが、24°C で栽培した L^{la}/L^{la} 植物は PaMMV-J に抵抗性を示した。

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