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Rapid Communication

TMV recombinants encoding fused foreign transmembrane domains to the CP subunit caused local necrotic response on susceptible tobacco

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Abstract

With regard to the effects of various foreign peptides fused to the coat protein subunits on the infectivity of corresponding TMV recombinants, some of TMV recombinants were found to induce necrotic local lesions on the inoculated leaves of susceptible tobacco. This paper reported that there existed a group of TMV recombinants in which the fused foreign peptides contained a transmembrane domain according to the predictions by three programs of SOSUI, TMpred and DAS. Further studies showed for the first time that a foreign transmembrane domain in a fused peptide of the corresponding TMV recombinant would result in the local lesions on the susceptible tobacco leaves. In addition, it was concluded that none of the TMV recombinants that systematically infected susceptible tobacco contained a transmembrane domain in the coat protein subunits.

Keywords: TMV; Transmembrane domain; Necrotic local lesion; Susceptible tobacco

Introduction

Tobacco mosaic virus (TMV), a type member of the Tobamovirus group, is one of the well-characterized plant viruses. In infected tobacco, the viral coat protein (CP) may constitute up to 7% of the total host protein in quantity and can be easily purified from plant tissue in the form of viral particles. TMV has been considered as a vector to express foreign proteins or short peptides in host plants due to its high replication efficiency and stability of virions in host plants. For expressing a short peptide, the coding sequence was fused *in-frame* to the CP coding sequence close to the C-terminus. The resulting recombinant CP subunits could function as the wild-type CP subunits in assembling viral genome to particles and the objective short peptides would be expressed on the surface of the particles (Lim et al., 2002; Bendahmane et al., 1999; Fitchen et al., 1995; Gilleland et al., 2000; Hamamoto et al., 1993; Koo et al., 1999; Staczek et al., 2000; Sugiyama et al., 1995; Takamatsu et al., 1990; Turpen et al., 1995). Such expressed short peptides were suggested to be useful for vaccine purpose, especially in stockbreeding, as the immunogenicity was highly enhanced when the CP subunit was injected into animals as an adjuvant (Bendahmane et al., 1999; Gilleland et al., 2000; Koo et al., 1999; Staczek et al., 2000; Wu et al., 2003).

However, unsuccessful expressions of certain short peptides by this strategy were often experienced: the corresponding recombinant TMV genomes became less infectious, and in some cases, they even induced local lesions on the inoculated leaves instead of the typical systematic mosaic symptoms seen on young leaves of the susceptible tobacco hosts such as Samsun nn and Xanthi nn (Bendahmane et al., 1999; Takamatsu et al., 1990). At present, little is known about the mechanisms that control the display of a foreign peptide on the surface of the recombinant TMV. It was ever proposed that the charge and isoelectric point of the recombinant CP subunit might play an important role in the successful display of a foreign peptide on the surface of TMV (Bendahmane et al., 1999). Here, we reported for the first time that a group of recombinant TMV genomes encoding short peptides with a transmembrane domain induced necrotic local lesions on susceptible tobacco *N. tabacum* Samsun nn and *N. tabacum*

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Xanthi nn while the transmembrane domain was adapted from various known proteins, such as the spike and membrane proteins of severe acute respiratory syndrome (SARS) coronavirus (Marra et al., 2003), the sodium-phosphate coupled transporter protein DvSPT1 of *Dunaliella viridis* (Li et al., 2006) and the voltage-gated sodium channel protein Scn2a of rat (Noda et al., 1986). Interestingly, further analysis led us to conclude that among the reported CP subunits encoded by infectious TMV recombinants, none contained any transmembrane domains.

Results

TMVS1241, TMVM109 and TMVM125 systematically infected Samsun nn tobacco

The in vitro transcripts specifying TMVS1241, TMVM109 and TMVM125 synthesized from pTMVS1241, pTMVM109 and pTMVM125, respectively, were inoculated onto the leaves of *N. tabacum* Samsun nn plants. Similar to that infected by wtTMV (Fig. 1), all the inoculated plants were infected, showing the typical mosaic symptoms (Fig. 1). The cDNA fragments specifying the fused S1241, M109 and M125 sequences could be amplified by RT-PCR analysis from young leaves of the corresponding infected tobacco plants (Fig. 2A, lanes 3, 4, 6), which were confirmed by DNA sequencing (data not shown). Abundant amount of the CP subunits of the three constructs accumulated in the young leaves similar to that of wtTMV (Fig. 2B, lane 2), which was shown by SDS-PAGE (Fig. 2B, lanes 3, 4, 6) and Western blot analysis (Fig. 2C, lanes 3, 4, 6). Using a standard procedure, TMVS1241, TMVM109 and TMVM125 particles (Fig. 2D, lanes 3, 4, 6) could be purified easily from the infected leaves with the yields similar to that of wtTMV (Fig. 2D, lane 2). Passage tests indicated that the inserted peptides were quite stable in the CP recombinants and no mutations occurred in the inserted region (data not shown).

An infectious mutant TMVM109mu was isolated from one tobacco plant inoculated with TMVM109 after a few passages, in which the F^{111} in the M109 region of the recombinant CP was mutated to S^{111} (from a nonpolar phenylalanine to a polar serine). The mutant remained infectious as efficiently as wtTMV and its cognate TMVM109 according to the symptom appeared on young leaves (Fig. 1), the yield of the purified virus particles (Fig. 2D, lane 5), RT-PCR (Fig. 2A, lane 5) and Western blot (Fig. 2C, lane 5) assays.

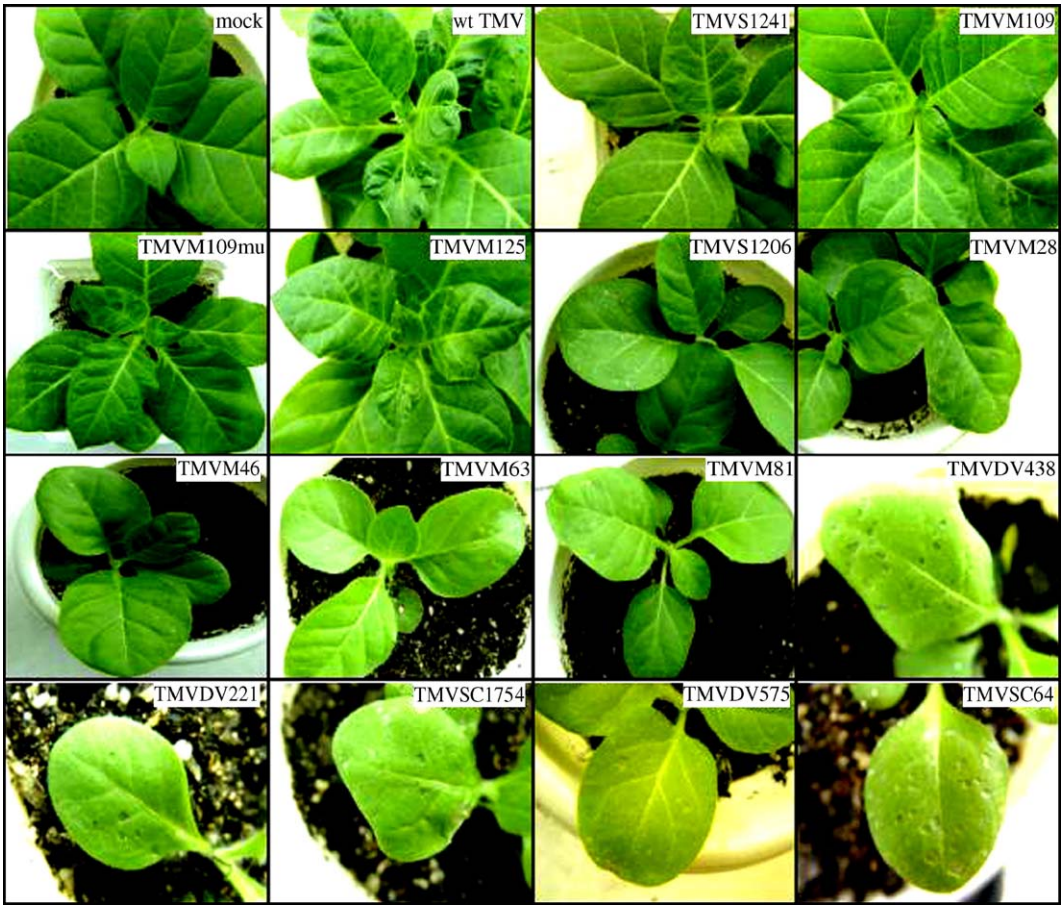

Fig. 1. Symptoms on Samsun nn leaves inoculated with transcripts of various recombinant TMV viruses. Four-week-old seedlings of *N. tabacum* Samsun nn were mechanically inoculated individually with the transcripts which were synthesized from various TMV recombinants. The plants infected by no virus (mock), wtTMV, TMVS1241, TMVM109, TMVM109mu and TMVM125 were photographed 3 weeks post-inoculation while the plants infected by TMVS1206, TMVM28, TMVM46, TMVM63, TMVM81, TMVDV438, TMVDV221, TMVSC1754, TMVDV575 and TMVSC64 were photographed 4 days post-inoculation.

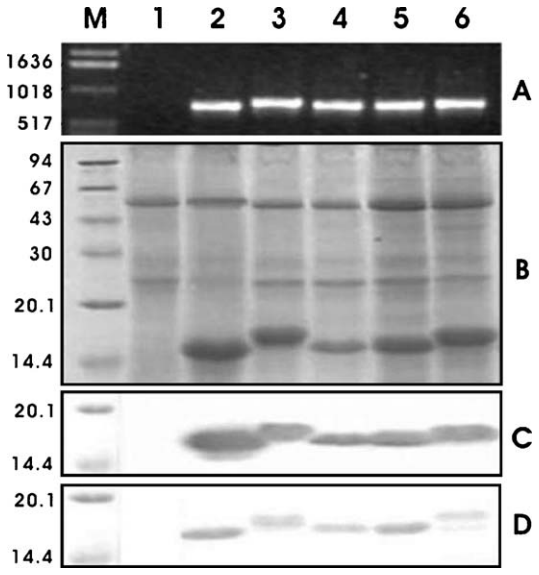

Fig. 2. Detection of TMVS1241, TMVM109, TMVM109mu and TMVM125 in tobacco plants. Samples collected from the tobacco leaves infected with no virus (lane 1), wtTMV (lane 2), TMVS1241 (lane 3), TMVM109 (lane 4), TMVM109mu (lane 5) and TMVM125 (lane 6) were processed for (A) RT-PCR using primers SQ3(+) and NSI(−), (B) 15% SDS-PAGE stained with Coomassie brilliant blue, (C) Western blot analysis using anti-TMV CP IgG and (D) 15% SDS-PAGE of CP subunits extracted from various purified recombinant virus particles and the gel were stained with Coomassie brilliant blue. The RT-PCR products were separated by 1.0% agarose and stained with ethidium bromide. M represents DNA 1 kb ladder (A) and protein molecular weight markers (B to D); the numbers listed on the left sides represent sizes of the markers (bp in panel A, and kDa in panels B to D).

TMVS1206 and TMVM28 caused necrotic local lesions on Samsun nn tobacco leaves

The in vitro transcripts specifying TMVS1206 and TMVM28 synthesized from the corresponding plasmids pTMVS1206 and pTMVM28 were inoculated onto the leaves of the tobacco seedlings of *N. tabacum* Samsun nn. Local lesions appeared on the inoculated leaves 3 to 4 days post-inoculation (Fig. 1), which resembled those seen in the hypersensitive response (HR) of resistant tobacco *N. tabacum* Samsun NN, whereas no systematic symptoms were observed on the young leaves after 2 weeks or longer period of the inoculation. Further RT-PCR and Western blot analysis indicted that the young leaves were not infected (data not shown).

A transmembrane domain existed in the CP subunits of TMVS1206 and TMVM28

The possible transmembrane domains in the recombinant CP subunits were predicted by three of the most currently used programs SOSUI, TMpred and DAS. As shown in Table 1, all the three programs indicated that there existed a possible transmembrane domain at the C-terminal regions of the CP subunits in TMVS1206 and TMVM28, but not in wtTMV, TMVS1241, TMVM109 and TMVM125. Thus, the transmembrane domain appeared to be related to the necrotic local lesions on the susceptible tobacco leaves.

Prediction of transmembrane domains in various recombinant TMV CP subunits

To validate our hypothesis, the sequences of the various foreign peptides in the recombinant TMV CP subunits (from our laboratory's data and other published data) were analyzed for possible transmembrane domains by the three programs mentioned above (Table 2). Based on the symptoms on the susceptible tobacco plants, there were two groups of TMV recombinants: systemic infection-related and necrotic local lesion-related. The former includes those encoding the fused peptides F11, F14 and F14mu from foot-and-mouth disease virus (FMDV) VP1 protein (Wu et al., 2003), F25 and F20 from FMDV VP1 protein (Jiang et al., 2006), ZP3 from *murine zona pellucida ZP3* protein (Fitchen et al., 1995), 9-14mer from *Pseudomonas aeruginosa* protein F (Gilleland et al., 2000; Staczek et al., 2000), G5.24 from rabies virus glycoprotein (Bendahmane et al., 1999), 5B19, 5B19L and RB19E from murine hepatitis virus (MHV) S-glycoprotein (Bendahmane et al., 1999; Koo et al., 1999), neuropeptide nocistatin mNST from bovine brain (Lim et al., 2002), ACEI inhibitor (Hamamoto et al., 1993), pep8 and pep18 from influenza virus hemagglutinin (HA) (Sugiyama et al., 1995), pep13 from human immunodeficiency virus type I envelop protein (Sugiyama et al., 1995), $(QGPGAP)_2$ from malaria (Turpen et al., 1995) and peptides S1241, M109, M109mu and M125 from severe acute respiratory syndrome (SARS) coronavirus spike and membrane protein (Marra et al., 2003) studied in this paper. The latter includes those encoding the fused peptidesRB19 (Bendahmane et al., 1999), ENK (Takamatsu et al., 1990), FN20, WD22, WQ22, FQ22-M and FQ22-I (unpublished data in our laboratory). However, predicted by the three programs, none of the above TMV recombinants contained any transmembrane domains in the corresponding CP subunit. This observation suggested that the TMV recombinants that caused typical systematic infections on susceptible tobacco plant contained no transmembrane domains in their recombinant CP subunits, however, to TMV recombinants that induced necrotic local lesions, a transmembrane domain in the recombinant CP subunits seemed not to be necessary.

Viruses containing a transmembrane domain in the recombinant CP subunits caused local lesions on Samsun nn tobacco leaves

According to the predictions by the SOSUI, TMpred and DAS programs, various foreign peptide sequences were selected from different protein sources, such as the SARS membrane proteins (Marra et al., 2003), the sodium-phosphate coupled transporter protein DvSPT1 from *Dunaliella viridis* (Li et al., 2006) and the animal voltage-gated sodium channel protein Scn2a from rat (Noda et al., 1986), to generate TMV recombinants TMVM46, TMVM63, TMVM81, TMVDV438, TMVDV221 and TMVSC1754, each of which contained a foreign transmembrane domain in the recombinant CP subunit (Table 1). All of the six recombinants induced the necrotic local lesions on the inoculated leaves of Samsun nn tobacco

Table 1
Transmembrane domains at the C-terminus of recombinant CP predicted by SOSUI, TMpred and DAS programs

Prediction method	Recombinant CP	Transmembrane domain[a]	Position[b]	Length
SOSUI	wt CP	–	–	0
	CP-S1206	SSSGLVW*LIAIVMVTILLCCMTS*	146–168	23
	CP-M28	ESSSGLVW*LAWIMLLQFAY*	145–163	19
	CP-M46	SSSGLVW*YIIKLVFLWLLWPVTL*	146–168	23
	CP-M63	SSSGLVW*CFVLAAVYRINWVTGG*	146–168	23
	CP-M81	SSSGLVW*IAMACIVGLMWLSYFV*	146–168	23
	CP-DV438	SSSGLVW*WILVIGAAGIVFGLAM*	146–168	23
	CP-DV221	SSSGLVW*AFFMIPVLMMITIFIV*	146–168	23
	CP-SC1754	SSSGLVW*FFFVSYIIISFLVVVN*	146–168	23
TMpred[c]	wt CP	–	–	0
	CP-S1206	VW*LIAIVMVTILLCCMTS*	151–168 (o→i)[d]	18
		GLVW*LIAIVMVTILLCCM*	149–166 (i→o)	18
	CP-M28	SSSGLVW*LAWIMLLQFAYSN*	146–165 (o→i)	20
		FESSSGLVW*LAWIMLLQFAYS*	144–164 (i→o)	21
	CP-M46	GLVW*YIIKLVFLWLLWPVTLA*	149–169 (o→i)	21
		GLVW*YIIKLVFLWLLWPVTLA*	149–169 (i→o)	21
	CP-M63	GLVW*CFVLAAVYRINWV*	149–165 (o→i)	17
		FESSSGLVW*CFVLAAVYRI*	144–162 (i→o)	19
	CP-M81	GLVW*IAMACIVGLMWLSYF*	149–167 (o→i)	19
		GLVW*IAMACIVGLMWLSYFV*	149–168 (i→o)	20
	CP-DV438	LVW*WILVIGAAGIVFGLAMYGY*	150–171 (o→i)	22
		GLVW*WILVIGAAGIVFGLAM*	149–168 (i→o)	20
	CP-DV221	W*AFFMIPVLMMITIFIVLL*	152–170 (o→i)	19
		W*AFFMIPVLMMITIFIVLL*	152–170 (i→o)	19
	CP-SC1754	VW*FFFVSYIIISFLVVV*	151–167 (o→i)	17
		VW*FFFVSYIIISFLVVV*	151–167 (i→o)	17
DAS	wt CP	–	–	0
	CP-S1206	LVW*LIAIVMVTILLCC*	150–165	16
	CP-M28	LVW*LAWIMLLQFAYSNRNRF*	150–169	20
	CP-M46	VW*YIIKLVFLWLLW*	151–164	14
	CP-M63	LVW*CFVLAAV*	150–159	10
	CP-M81	VW*IAMACIVGLMWLS*	151–165	15
	CP-DV438	LVW*WILVIGAAGIVF*	150–164	15
	CP-DV221	VW*AFFMIPVLMMITIF*	151–166	16
	CP-SC1754	LVW*FFFVSYIIISFLVV*	150–166	17

[a] The amino acids corresponding to TMV CP and foreign peptides are underlined and italicized, respectively.
[b] The position numbers are based upon the sequence of the recombinant CP.
[c] Predicted with the amino acid length of minimal 17 and maximal 33 of the hydrophobic part of the transmembrane domain.
[d] i→o and o→i represents inside to outside and outside to inside helices predicted by TMpred.

plants 3 to 4 days post-inoculation (Fig. 1), which resembled those seen in the hypersensitive response (HR) of resistant tobacco. In addition, no distinct systematic symptoms were observed on the young leaves after 2 weeks or longer period of inoculation (Fig. 1), and viral RNA and coat protein were also not detected from the young leaves.

The observations led us to further suggest that the recombinant TMV encoding a C-terminal transmembrane domain in the recombinant CP subunit caused local lesions on susceptible tobacco leaves, although some TMV recombinants that contained no C-terminal transmembrane domain in the CP subunits also caused necrotic local lesions.

Possible effects of the content of the hydrophobic amino acid residues in the fused foreign peptides on the viral infection

The percentage of the hydrophobic amino acid residues in each foreign peptide encoded by the above TMV recombinants was listed in Table 2. The peptides from SC64 to DV221 contained 60.0% to 94.4% of the hydrophobic amino acid residues, and all of the corresponding TMV recombinants such as those encoding foreign peptides SC64, S1206, M63, M28, DV575, DV438, M81, SC1754, M46 and DV221 caused local lesions on the inoculated susceptible tobacco leaves (Fig. 1), except that the TMV recombinant encoding ACE1 systematically infected the tobacco plants and resulted in the typical mosaic symptoms. On the other hand, the peptides from FN20 to RB19 contained much lower percentages of the hydrophobic residues (35.0% to 36.4%), and all of them induced local lesions on the inoculated leaves of the susceptible tobacco. It seemed that there existed no clear relationships between the percentages of the hydrophobic amino acid residues in the foreign peptides and the induction of the necrotic local lesions on the susceptible tobacco.

Discussion

Display of foreign peptides on the surface of TMV has been proven to be an effective way to produce valuable

Table 2
Summary of the foreign peptides fused to the CP subunits of the various TMV recombinants

Peptide name	Fused peptide			Recombinant CP		Symptoms[a] on	
	Peptide sequence	p*I*[b]	(%)[c]	p*I*/net charge[b]	Transmembrane domain[d]	Inoculated leaves	Young leaves
wt	–	–	–	5.09/−2	–	–	M
pep8	CKRGPDSG	8.22	12.5	5.44/−1	–	–	M
ZP3	CSSSSNGHPQFQR	8.26	15.4	5.48/−1	–	–	M
9-14mer	TDAYNQKLSERRAN	8.59	21.4	5.48/−1	–	–	M
M109mu	WSSNPETN	4.00	25.0	4.92/−3	–	–	M
mNST	IEGRAEPGADDAEEVEQKQLQ	4.00	28.6	4.62/−7	–	–	M
5B19L	PLLGCIGSTCAEDGN	3.84	33.3	5.01/−4	–	–	M
RB19E	RLLGCIGSTCAE	6.42	33.3	5.34/−2	–	–	M
FN20	ETQVQRRQHTDVSFILDRFV	6.76	35.0	5.41/−1	–	LL	–
FQ22-M	FQPRPQMHNDGDFEEIPEEYLQ	4.07	36.4	4.68/−6	–	LL	–
FQ22-I	FQPRPQIHNDGDFEEIPEEYLQ	4.07	36.4	4.68/−6	–	LL	–
WD22	WDPRPQRHNDGDFEPIPEEYLQ	4.29	36.4	4.77/−5	–	LL	–
RB19	RLLGCIGSTCA	8.07	36.4	5.42/−1	–	LL	–
M109	WSFNPETN	4.00	37.5	4.92/−3	–	–	M
pep13	KSRIQRGPGRAFV	–	38.5	8.94/+ 2	–	–	M
pep18	SKAFSNCYPYDVPDYASL	4.21	38.9	4.92/−3	–	–	M
5B19	LLGCIGSTCA	5.94	40.0	5.31/−2	–	–	M
WQ22	WQPRPQIHNDGDFEPIPEEYLQ	4.17	40.9	4.75/−5	–	LL	–
S1241	DDSEPVLKGVKL	4.56	41.7	4.96/−3	–	–	M
F14mu	RHEQKIVAPVKQTL	9.99	42.9	6.81/+ 1	–	–	M
F14	RHKQKIVAPVKQTL	11.26	42.9	8.86/+ 3	–	–	M
G5.24	PPDQLVNLHDFRSDEIEHLVVEE	4.40	43.5	4.92/−8	–	–	M
F25	RHKQKIVAPVKQTLPNVRGDLQVLA	11.10	48.0	8.86/+ 3	–	–	M
$(QGPGAP)_2$	QGPGAPQGPGAP	5.52	50.0	5.09/−2	–	–	M
ENK	MYGGFL	5.52	50.0	5.09/−2	–	LL	–
M125	GTIVTRPLME	6.00	50.0	5.13/−2	–	–	M
F11	PNVRGDLQVLA	5.84	54.5	5.11/−2	–	–	M
F20	VPNLRGDLQVLAQKVARTLP	10.83	55.0	6.50/0	–	–	M
SC64	FIYGDIPPEMVSEPL	3.57	60.0	4.69/−5	–	LL	–
S1206	LIAIVMVTILLCCMTSCC	5.50	61.1	5.09/−2	+	LL	–
M63	CFVLAAVYRINWVTGGIA	8.22	61.1	5.42/−1	+	LL	–
M28	LAWIMLLQFAYSNRNRFL	10.83	61.1	6.49/0	+	LL	–
DV575	SMQALLDRCAAAPAP	5.83	66.7	5.11/−2	–	LL	–
DV438	WILVIGAAGIVFGLAMYGY	5.52	68.4	5.09/−2	+	LL	–
ACEI	FFVAPFPKVFGK	11.17	69.2	8.09/0	–	–	M
M81	IAMACIVGLMWLSYFVASFR	8.22	70.0	5.42/−1	+	LL	–
SC1754	FFFVSYIIISFLVVVNMY	5.52	72.2	5.09/−2	+	LL	–
M46	YIIKLVFLWLLWPVTLA	8.59	88.2	5.42/−1	+	LL	–
DV221	AFFMIPVLMMITIFIVLL	5.52	94.4	5.09/−2	+	LL	–

[a] Estimated using the program Vector NTI suite.
[b] The percentage of the hydrophobic amino acid residues in the total foreign peptide.
[c] Predicted by SOSUI, TMpred and DAS programs; +: found, −: not found.
[d] Symptoms on *N. tabacum* Samsun nn or Xanthi nn plants: LL, local lesion; M, mosaic systematic symptoms; −, no symptoms.

peptide medicines using tobacco plant. However, we have encountered some foreign peptides that severely affected the viral infectivity, viral particle formation and even the symptom appearance on susceptible hosts (Bendahmane et al., 1999; Porta et al., 1994; Takamatsu et al., 1990). It has been suggested that various criteria such as the amount of the amino acid residues (Wu et al., 2003), p*I*/charge value (Bendahmane et al., 1999) of foreign peptides and fusion sites (Fitchen et al., 1995; Hamamoto et al., 1993; Sugiyama et al., 1995; Takamatsu et al., 1990; Turpen et al., 1995) in the TMV CP correlate with the infectivity and stability of the recombinant TMV in tobacco plants. Unfortunately, to our knowledge, there is no such single criterion that could simply correlate to the host response to the virus infection. It indeed has been very difficult for us to further investigate the host–virus interactions.

In our studies, we have experienced that many unknown factors would result in the local lesions on susceptible tobacco plant by various TMV recombinants. In this paper, we looked into, for the first time, a possible relationship between the transmembrane domain in the CP subunits and the induction of the local lesions on susceptible tobacco plants, and concluded that the transmembrane domain at the C-terminus of the recombinant CP subunit in the region of the fused foreign peptide would determine the corresponding TMV recombinant to induce the necrotic local lesion response on the inoculated leaves of Samsun nn tobacco plant as well as Xanthi nn tobacco (data not shown); we also concluded that none of the

reported infectious TMV recombinants contained any transmembrane domains in the CP subunits. The transmembrane domains involved in this report were adapted from different proteins and organisms and shared no homologies in the sequences to each other (data not shown); however, all the corresponding TMV recombinants caused necrotic local lesions on the inoculated leaves of the Samsun nn and Xanthi nn (data not shown) tobacco plants.

The percentage of the hydrophobic amino acid residues is usually high in the transmembrane domains; however, our results clearly indicate that the percentage of the hydrophobic residues has no relationships with the necrotic local lesions.

Our results also led us to further believe (Wu et al., 2003) that there is no clear causality of the p*I*/charge value of the recombinant CP to the infection of the TMV recombinants on the susceptible tobacco although the importance of successful display of foreign peptides on the surface of TMV recombinants was ever emphasized (Bendahmane et al., 1999). As shown in Table 2, some exceptional examples such as TMVF14 (Wu et al., 2003) and TMVF25 (Jiang et al., 2006) with the p*I*/charge values of 8.86/+3 could systematically infected Samsun nn plants, and had no mutations in the entire CP region in its progenies. Some other exceptional examples, such as those encoding CP-fused FQ22-M, FQ22-I, WD22, WQ22 and SC64 with p*I*/charge values of 4.68/−6, 4.68/−6, 4.77/−5, 4.75/−5 and 4.69/−5 (Table 2), all caused local lesions on *N. tabacum* Samsun nn.

TMV induces necrotic local lesions on *N. tabacum* Samsun NN as a result of the *N* resistance gene (Whitham et al., 1994) and an uncharacterized interaction with TMV replicase (Abbink et al., 1998; Padgett and Beachy, 1993; Padgett et al., 1997). The *N* gene-mediated HR is temperature sensitive, the resistance to TMV occurred only at temperatures below 30 °C (Weststeijn, 1981); however, our unpublished data indicated that the TMV recombinants encoding foreign peptides M63, M81 and FN20 (Table 1) could induce necrotic local lesions on Samsun NN at both 25 °C and 32 °C (data not shown). However, the lesions caused by the TMV recombinants encoding a fused transmembrane domain appeared at the same time as wtTMV did on Samsun NN, albeit in some cases, the lesions appeared about 1 day later than wtTMV. The size, color and the amount of the lesions caused by this group of the TMV recombinants were similar to wtTMV (data not shown).

The resistance of *Nicotiana sylvestris* carrying *N′* gene was known to be elicited by some TMV mutants with structural alterations in the CP subunits that could interact with an uncharacterized host receptor to elicit the HR (Culver et al., 1994). The necrotic local lesions of *N. tabacum* Samsun nn and *N. tabacum* Xanthi nn described in this report appeared to be similar with the HR on tobacco carrying *N′* gene, but an unknown mechanism related to this necrotic local lesion response of the susceptible tobacco to these TMV recombinants could not be ruled out. Our observation would provide a potential system for studying the mechanisms of virus–host interactions.

For practical purposes, our observation strongly demonstrates that any possible transmembrane sequence in foreign peptide must be avoided in the mass expression of foreign peptides in tobacco plant using this type of the TMV-based vector.

Materials and methods

Plasmid construction and in vitro transcription

The plasmids pTMVS1241, pTMVM109, pTMVM125, pTMVS1206, pTMVM28, pTMVM46, pTMVM63, pTMVM81, pTMVDV438, pTMVDV221, pTMVDV575, pTMVSC1754 and pTMVSC64 specifying various TMV recombinants were generated by a two-step PCR procedure described by Wu et al. (2003). The corresponding recombinant CP genes in these plasmids contained sequences specifying the foreign peptides of S1241(D^{1241}-L^{1252}), M109(W^{109}-N^{116}), M125(G^{125}-E^{134}), S1206(L^{1206}-C^{1223}), M28(L^{28}-L^{45}), M46(Y^{46}-A^{62}), M63(C^{63}-A^{80}), M81(I^{81}-R^{100}), DV438(W^{438}-Y^{456}), DV221(A^{221}-L^{238}), DV575(S^{575}-P^{589}), SC1754(F^{1754}-Y^{1771}) and SC64(F^{64}-L^{78}) as shown in Table 2. The various peptides were respectively fused to the C-terminus of CP at the site of Trp^{152} and the recombinant CP subunits lacked the terminal peptides Thr^{153}-Thr^{158} of CP due to early translation termination by an inserted TAG codon.

Inoculation of plants and virus purification

Transcripts from plasmids linearized by *Pst*I were mechanically inoculated onto the leaves of 4-week-old *N. tabacum* Samsun nn and Xanthi nn plants. The inoculated tobacco plants were cultured in a greenhouse with a 16 h photoperiod. The systematically infected leaves were harvested and processed for virus purification. The purified virus was suspended in 50 mM phosphate buffer (pH 7.2), 1 mM EDTA and stored at 4 °C.

RT-PCR

Total RNA extracted from 1 leaf disk (1 cm in diameter) with 500 μl Trizol reagent was subjected to RT-PCR using the primers NSI (−) and SQ3 (+) as described previously (Wu et al., 2003). The products were subjected to DNA sequencing for any possible mutations in the fused CP genes.

SDS-PAGE and Western blot analysis

Total proteins were extracted from 1 leaf disk for 15% SDS-PAGE and Western blot analysis as described previously (Wu et al., 2003).

Transmembrane domain prediction

The transmembrane domains in the recombinant CP subunits were predicted by SOSUI (Hirokawa et al., 1998), TMpred (Hofmann and Stoffel, 1993) and DAS (Cserzo et al., 1997), respectively. The predicted results for each CP subunit listed in this report were confirmed in agreement by all of the three programs.

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