Helper Component Mutations in Nonconserved Residues Associated with Aphid Transmission Efficiency of a Pepper Isolate of Potato Virus Y

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ABSTRACT

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The aphid transmission properties of a pepper isolate of potato virus Y belonging to the pathotype 1-2 (PVY 1-2) have been characterized. PVY 1-2 was not transmitted in plant-to-plant experiments, although purified virus particles were efficiently transmitted when supplemented with heterologous helper component (HC) of the transmissible isolate PVY 0 AT through membrane acquisition assays, indicating that its coat protein

was functional in transmission. Additionally, virions of PVY 1-2 were able to bind to different HCs in *in vitro* binding assays. Analysis of the sequence of the PVY 1-2 HC gene and comparison with that of PVY 0 AT revealed 19 nucleotide differences, but only 2 resulted in amino acid changes, one of which induced a change of charge. Neither of these two amino acid changes occurred within the cysteine-rich domain, nor did they coincide with conserved motifs of the HC protein known to be involved in aphid transmission and which are present in all known potyviruses. However, both changes are located in positions highly conserved among PVY strains. The possible role of both mutations on the activity of the PVY 1-2 HC in aphid transmission is discussed.

Potato virus Y (PVY) is the type member of the genus *Potyvirus* (family *Potyviridae*), the largest group of RNA plant viruses (17, 18). PVY consists of a complex group of strains and pathotypes which have been identified and classified according to their biological properties and that cause serious losses in many important crops all over the world, including pepper (9). PVY isolates infecting pepper (*Capsicum annuum* L.) have been classified into three pathotypes (types 0, 1, and 1-2) based on their ability to overcome certain resistance genes of pepper to PVY infection (12).

Nonpersistent aphid transmission of potyviruses requires, in addition to virions, a virus encoded non-structural protein, "helper component" (HC), which is essential for virion retention in the stylets of the aphid (2,33). This protein has been speculated to act as a bridge between the coat protein (CP) of the virus particle and a putative receptor in the aphid mouthparts (23). A number of potyvirus strains which are naturally nontransmissible by aphids due to alterations in the amino acid sequence of the HC have been reported (8,13,20,30). In addition, mutational analysis has proved that two highly conserved motifs in the HC sequence are directly involved in the activity of this protein during the transmission process. Substitution of Lys to Glu or to other amino acids besides Arg in the highly conserved Lys-Ile-Thr-Cys (KITC) sequence at the N-terminus of the HC of tobacco vein mottling virus (TVMV; 3,4) and Lys to Glu in the KITC box of tobacco etch virus (TEV; 5) resulted in loss of HC activity. The KITC box, which lies in a conserved cysteine-rich region, has been suggested to be required for the interaction of HC with aphid stylets (5). Certain mutations in the conserved Pro-Thr-Lys (PTK) motif at the central region of the HC of zucchini yellow mosaic virus (ZYMV) recently have been shown to result in loss of aphid transmission and virion-HC

binding (21). Additionally, a Phe to Leu replacement at amino acid 10 of the HC of TEV also renders HC nonfunctional (5). The knowledge of the domains or amino acid residues involved in the activity of the HC during the transmission process might facilitate the design of new disease control strategies for preventing virus spread by aphids in nature.

We have previously characterized the aphid transmission properties of pepper pathotypes PVY 0 and PVY 1 (8). In the present work, we analyzed the aphid transmission properties of a PVY isolate belonging to pepper pathotype 1-2 and determined the complete nucleotide and predicted amino acid sequence of its HC.

MATERIALS AND METHODS

Virus isolates. The isolate P-22-88, belonging to the PVY pepper pathotype 1-2 and originating from Spanish field-grown pepper plants, was used in this study. This isolate was classified and provided by M. P. Luis-Arteaga (SIA-DGA, Zaragoza, Spain) and was maintained in *C. annuum* L. 'Yolo Y,' *Nicotiana tabacum* L. 'Xanthi nc,' and *N. sylvestris* through repeated mechanical passages. P-22-88 was designated here, for simplicity, as PVY 1-2. The aphid-transmissible isolate PVY 0 AT and the non-aphid transmissible isolate PVY 1 were used in previous studies (8).

Purified virus and HC preparation. Viruses and HCs were purified from leaves of systemically infected tobacco plants 3 weeks after inoculation and assayed for biological activity as described (19,24). Virus concentrations were determined spectrophotometrically and HC preparations were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% polyacrylamide gels and electroblotted onto nitrocellulose following the method of Towbin et al. (31). PVY HC monoclonal antibodies (MAbs) were used for HC immunodetection (7). HC preparations were shown to be virus-free (data not shown) by membrane acquisition tests (described below) and mechanical inoculation on tobacco plants.

Aphid transmission assays. The procedures for rearing and handling the aphids (*Myzus persicae* Sulzer) as well as the transmission assays, either by *in vivo* acquisition from plants or in

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vitro through Parafilm membranes, have been described elsewhere (22). Acquisition access feeding was for 5 to 10 min, and inoculation access feeding was overnight. Aphids were killed by spraying with an insecticide, and the plants were placed in a growth room. Plant infection was confirmed after 2 or 3 weeks by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using MAbs to PVY (Ingenasa, Madrid). Purified viruses and partially purified HCs from PVY 1-2 and PVY 0 AT used in membrane transmission tests were adjusted to equal concentrations by western blot analysis to compare the level of aphid transmission of both isolates.

Dot-blot binding assay. The binding reaction between HC and PVY 1-2 virions was conducted according to Blanc et al. (5). Partially purified PVY 1-2 HC and PVY 0 AT HC were blotted on nitrocellulose membranes to be incubated with purified PVY 1-2 virions at a concentration of 3 µg/ml. Blots were successively probed with a polyclonal anti-PVY virion antibody, with goat anti-rabbit peroxidase conjugate and chromogenic substrate. As a control, purified TEV HC (6) was also dot blotted on nitrocellulose membrane and processed as described above.

Cloning and sequencing of the PVY 1-2 HC gene. Amplification of the PVY 1-2 HC-encoding region was carried out by immunocapture (34) followed by two-step reverse-transcriptase polymerase chain reaction (RT-PCR; 8). Immunocapture of clarified PVY 1-2-infected tobacco extract was performed directly in the tubes used for the RT-PCR reactions, which were previously coated with PVY polyclonal antibodies. The following PCR amplification scheme was used: denaturation for 5 min at 92°C; 25 cycles of 30 s at 92°C, 1 min at 52°C, and 1 min and 30 s at 72°C; and a final 5-min elongation step at 72°C. The downstream HC1 (5' ACCAACTCTATAGTGTTC 3') and upstream HC2 (5' CGTTC-CAAAGTCACTC 3') oligonucleotide primers at positions 2404 and 992, respectively, were designed according to the genomic sequence of the PVY 0 and PVY N isolates (8,26). Amplified cDNA fragments were purified from agarose gels using GeneClean (Bio 101, Vista, CA) and directly cloned into a PCR fragment cloning vector pGEM-T Easy (Promega Corp., Madison, WI). Recombinant plasmids were used to transform competent *Escherichia coli* DH5α. Positive clones were tested by restriction enzyme analysis of small-scale preparations of plasmid DNA. The nucleotide se-

quence of the PVY 1-2 HC gene was obtained by means of an ABI PRISM sequencer (Applied Biosystems, Foster City, CA) with purified plasmids as templates. HC1, HC2 and the internal upstream HC3 primer (5' GCAAGCACCATTCAAG 3') were used for PVY 1-2 HC sequencing. Each nucleotide determination was performed on three independent clones and directly twice on cDNA obtained by RT-PCR from viral RNA. Nucleotide and amino acid sequences from PVY 1-2 and PVY 0 AT HCs were aligned and compared, using CLUSTAL W, version 1.5 (28), with the following PVY strains and potyviruses: PVY 0 AT, PVY 1 (8), PVY C (30), PVY H (V. Thole, T. Dalmay, J. Burgyn, and E. Balazs, unpublished data; GenBank accession no. M95491), PVY N (26), plum pox virus (PPV; 14), TEV (1), turnip mosaic virus (TuMV; 32), TMV (10), and ZYMV (25).

RESULTS

Aphid transmission. In plant-to-plant experiments, transmission of PVY 1-2 was always negative regardless of the source of inocula (0% of 60 assays), whereas PVY 0 AT was efficiently transmitted (87% of 64 assays; Table 1). To determine whether the PVY 1-2 CP or HC were involved in this lack of transmissibility, a series of membrane acquisition transmission experiments were conducted with either homologous or heterologous combinations of purified virus and HC from PVY 0 AT and PVY 1-2.

Unlike the HC of PVY 1 (8), PVY 1-2 and PVY 0 AT HCs were readily partially purified from infected plants following standard procedures. Electrophoretic mobility, reactivity with PVY MAbs, and yield of purified HC were similar in PVY 1-2 and PVY 0 AT,

TABLE 1. Efficiency of plant-to-plant transmission of pepper isolates of potato virus Y (PVY) pathotypes 1-2 and 0 by aphids

Pathotype	Aphid transmission ^a				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Total
PVY 1-2	0/15	0/15	0/15	0/15	0/60
PVY 0 AT	14/16	14/18	13/15	15/15	56/64

^a Aphids (*Myzus persicae*) were starved for 2 to 3 h and allowed to feed for 5 to 10 min on virus-infected leaves. A total of 10 aphids per assay plant was used. Values are transmission frequencies based on number of infected plants/total number of tested plants in each experiment.

TABLE 2. Efficiency of membrane transmission by aphids of purified pepper isolates of potato virus Y (PVY) pathotypes 1-2 and 0 in the presence of homologous or heterologous helper components (HC)^a

Source of HC	HC dilution	PVY 1-2 transmission			PVY 0 transmission		
		Frequency	%	Range (%)	Frequency	%	Range (%)
PVY 1-2	1:2	7/50	14	0-27	6/70	9	0-20
	1:5	6/70	9	0-7	2/53	4	0-7
	1:10	10/120	8	0-27	8/88	9	0-27
PVY 0 AT	1:2	25/27	93	84-100	28/30	94	87-100
	1:5	24/27	89	87-92	... ^b	...	...
	1:10	24/30	80	74-87	...	...	...

^a Aphids (*Myzus persicae*) were starved for 2 to 3 h and allowed to feed for 5 to 10 min through stretched Parafilm membranes on a solution containing purified virions (50 µg/ml) mixed with partially purified HC in 0.1 M Tris-H₂SO₄, pH 7.2, 20 mM MgSO₄, and 20% sucrose. A total of 10 aphids per assay plant was used. Transmission frequencies are indicated by number of infected plants/total number of tested plants and are the combined results of at least three independent experiments with two different HC preparations.

^b Not performed.

Fig. 1. Binding assay between different helper components (HCs) and virions of pepper isolate pathotype 1-2 of potato virus Y (PVY). Partially purified PVY 1-2 and PVY 0 AT HCs and purified tobacco etch virus (TEV) HC were dot-blotted on nitrocellulose. Blots were incubated with purified PVY 1-2 virions (panel 1) or buffer control (panel 2) and successively probed with antibodies to PVY virions, goat anti-rabbit peroxidase conjugate, and chromogenic substrate.

S	N	A	E	N	F	W	K	G	L	D	N	N	W	A	R	M	R	Y	P	20
TCA	AAT	GCT	GAG	AAT	TTT	TGG	AAG	GGT	TTG	GAC	<u>AA</u> C	AAC	TGG	GCA	CGA	ATG	AGA	TAC	CCT	60
S	D	H	T	C	I	A	G	L	P	V	E	D	C	G	R	V	A	A	L	40
<u>TC</u> A	GAT	CAT	ACA	TGT	ATA	GCT	GGT	CTA	CCA	GTT	GAG	GAC	TGC	GGT	AGG	GTC	GCT	GCA	TTG	120
M	T	H	S	I	L	P	C	Y	K	M	T	C	P	T	C	A	Q	Q	Y	60
ATG	ACA	CAC	AGC	ATT	TTG	CCA	TGT	TAC	AAG	ATG	ACC	TGC	CCC	ACC	TGT	GCT	CAA	CAA	TAT	180
A	N	L	P	T	S	D	L	F	K	L	L	H	K	H	A	R	D	G	L	80
<u>GCC</u>	AAC	TTG	CCA	ACC	AGT	GAT	TTG	TTC	AAG	CTG	TTG	<u>CAC</u>	AAA	CAT	GCA	AGG	GAC	GGT	TTG	240
S	R	L	G	S	D	K	D	<u>Q</u>	F	V	H	V	S	K	F	L	E	T	L	100
AGT	CGA	TTG	GGA	TCA	GAT	AAA	GAT	<u>CAA</u>	CTC	GTG	CAT	GTT	AGT	AAG	TTC	TTG	GAA	ACG	TTG	300
E	H	L	T	E	P	V	D	L	N	L	E	L	F	N	E	I	F	K	S	120
GAG	CAT	TTA	ACT	GAA	CCA	GTT	GAC	TTA	AAT	CTC	GAG	CTT	<u>TTT</u>	AAT	GAG	ATA	TTC	AAA	TCC	360
I	G	E	K	Q	Q	A	P	F	K	N	L	N	V	L	N	N	F	F	L	140
ATA	GGG	GAG	AAG	CAG	CAA	GCA	CCA	TTC	AAG	AAT	TTG	AAT	GTC	TTA	AAC	<u>AAC</u>	TTC	TTT	TTG	420
K	G	K	E	N	T	A	H	E	W	Q	<u>I</u>	A	Q	L	S	L	L	E	L	160
AAA	GGA	AAA	GAA	AAT	ACA	GCT	CAT	GAA	TGG	CAG	<u>ATA</u>	GCT	CAA	TTG	AGT	TTG	CTC	GAA	TTA	480
S	R	F	Q	K	N	R	T	D	N	I	K	K	G	D	I	S	F	F	R	180
TCA	AGA	TTC	CAG	AAG	AAT	AGA	ACT	GAT	AAT	ATA	AAG	AAA	GGT	GAC	ATA	TCT	TTC	TTC	AGG	540
N	K	L	S	A	K	A	N	W	N	L	Y	L	S	C	D	N	Q	L	D	200
AAC	AAA	TTG	<u>TC</u> A	GCC	AAA	GCA	AAC	TGG	AAT	<u>CT</u> G	TAT	TTA	TCG	TGC	GAC	AAT	CAG	TTG	GAT	600
K	N	A	N	F	L	W	G	Q	R	E	Y	H	A	K	R	F	F	S	N	220
AAA	AAT	GCA	AAC	TTC	CTG	TGG	GGA	CAA	AGG	GAG	TAT	CAT	GCT	AAG	CGG	TTT	TTC	TCA	AAC	660
F	F	E	E	V	D	P	T	K	G	Y	S	A	Y	E	I	R	K	H	P	240
TTC	TTT	GAG	GAA	GTT	GAT	CCA	ACA	AAG	GGA	TAC	TCA	GCA	TAT	GAA	ATC	CGC	AAG	CAT	CCG	720
N	G	T	R	K	L	S	I	G	N	L	V	V	P	L	D	L	A	E	F	260
AAT	GGA	ACA	AGG	AAG	CTC	TCA	ATT	GGT	AAC	TTA	GTT	GTC	CCA	CTT	GAT	TTA	GCT	GAA	TTT	780
R	Q	R	M	K	G	D	Y	R	K	Q	P	G	V	S	K	K	C	T	S	280
AGA	CAG	AGG	ATG	AAA	GGT	GAC	TAT	AGG	AAA	CAA	CCA	GGG	GTC	AGC	AAA	AAG	TGC	ACA	<u>AGT</u>	840
S	K	D	G	N	Y	V	Y	P	C	C	C	T	T	L	D	D	G	S	A	300
TCG	AAA	GAT	GGT	AAT	TAT	GTA	TAT	CCT	TGT	<u>TG</u> C	TGC	<u>ACA</u>	ACA	CTT	GAT	GAT	GGT	TCA	GCC	900
I	E	S	T	F	Y	P	P	T	K	K	H	L	V	I	G	N	S	G	D	320
ATT	GAA	TCA	ACA	TTC	TAT	CCG	CCA	ACT	AAA	AAG	CAT	CTT	GTA	ATA	GGC	AAT	AGT	GGT	<u>GAC</u>	960
Q	K	Y	V	D	L	P	K	G	D	S	E	M	L	Y	I	A	K	Q	G	340
CAG	AAA	TAT	GTT	GAT	TTA	CCA	AAA	GGT	GAT	TCG	GAG	ATG	<u>TTG</u>	TAC	ATC	GCC	AAG	CAA	GGT	1020
Y	C	Y	I	N	V	F	L	A	M	L	I	N	V	S	E	E	D	A	K	360
TAC	TGC	TAT	ATC	AAC	GTG	TTT	CTT	GCA	ATG	CTA	ATT	AAC	GTT	AGC	GAG	GAG	GAC	GCA	AAG	1080
D	F	T	K	K	V	R	D	M	C	V	P	K	L	G	T	W	P	T	M	380
GAT	TTC	ACA	AAG	AAA	GTT	CGT	GAC	ATG	TGT	GTG	CCA	AAG	CTT	GGA	ACC	TGG	CCA	ACT	ATG	1140
M	D	L	A	T	T	C	A	Q	M	R	I	F	Y	P	D	V	H	D	A	400
ATG	GAT	TTG	<u>GCG</u>	ACT	ACT	TGT	GCT	CAA	ATG	AGA	ATA	TTC	TAC	CCT	GAC	GTG	CAC	GAT	GCG	1200
E	L	P	R	I	L	V	D	H	D	T	Q	T	C	H	V	V	D	S	F	420
GAA	TTG	CCC	AGA	ATA	TTA	<u>GT</u> T	GAC	CAT	GAT	ACT	CAG	ACG	TGT	CAC	GTG	GTT	GAC	TCT	TTT	1260
G	S	Q	T	T	G	Y	H	I	L	K	A	S	S	V	S	Q	L	I	L	440
GGT	TCG	CAA	ACA	ACT	GGG	TAC	CAT	ATT	CTA	AAA	GCA	<u>TC</u> T	AGT	GTG	TCT	CAA	CTC	ATC	TTG	1320
F	A	N	D	E	L	E	S	D	I	K	H	Y	R	V	G					456
TTT	GCA	AAT	GAT	GAG	TTA	<u>GAA</u>	TCT	GAT	ATA	AAA	CAC	TAT	AGA	GTT	GGT					1368

Fig. 2. Nucleotide sequence of the helper component (HC) gene of pepper isolate pathotype 1-2 of potato virus Y (PVY) and its deduced amino acid sequence. Nucleotide differences between PVY 1-2 and PVY 0 AT HCs are underlined on the PVY 1-2 sequence. Amino acid differences are boxed. Numbers to the right show the position from the N terminus of the HC.

as judged by western blot analysis (data not shown). Unexpectedly, in vitro membrane transmission of either PVY 1-2 or PVY 0 virions complemented with PVY 1-2 HC was successful, but with low efficiency (Table 2). Therefore, the PVY 1-2 HC seemed to be ineffective in promoting high rates of transmission of either of these isolates when tested at a series of dilutions in membrane-acquisition experiments (Table 2). In contrast, PVY 0 AT HC was able to assist the transmission of PVY 1-2 and PVY 0 AT at high rates (Table 2), indicating that PVY 1-2 virions can be efficiently transmitted when supplemented with an active HC, thus suggesting that a defect in the HC protein, rather than in the CP, of the PVY 1-2 might cause this deficiency in the virus transmission. On the other hand, no infection was shown in membrane aphid transmission experiments performed with partially purified HC alone, ruling out virus contamination.

Additionally, the lack of transmission of PVY 1-2 from plants apparently cannot be attributed to a low titer of virus or HC in the plant because the amounts of viral CP and HC as determined by SDS-PAGE and western blot, respectively, from purified preparations were as high as those from PVY 0 AT (data not shown).

Binding of PVY 1-2 virions to HCs. In vitro binding assays were performed to elucidate whether the low efficiency of aphid transmissibility of PVY 1-2 was due to inability of virions to interact with HC. PVY 1-2 virions were able to consistently bind to HC proteins from PVY 1-2, PVY 0 AT, and TEV that were dot blotted onto nitrocellulose membranes following the protocol of Blanc et al. (6; Fig. 1). The weak positive reactions recorded in the buffer-incubated membranes were due to low traces of contaminating CPs in the PVY HC preparations.

Sequence analysis of the PVY 1-2 HC. The CP of PVY 1-2 was functional in aphid transmission but its HC was unable to transmit efficiently either PVY 1-2 or PVY 0 AT in membrane acquisition experiments; therefore, we determined the nucleotide sequence of the PVY 1-2 HC gene (Fig. 2) and compared it with that of PVY 0 AT, already published (8), to find out which amino acid changes,

if any, might lead to a partial loss of activity of the PVY 1-2 HC during the transmission process. The deduced amino acid sequences also were compared with those of other strains of PVY and other potyviruses (Figs. 3 and 4).

Of the 19 nucleotide sequence differences found between the HC of PVY 0 AT and PVY 1-2, 17 did not involve amino acid changes; the remaining 2 changes induced amino acid substitutions (Fig. 2), indicating that either of these amino acid changes, or both, might be candidates involved in the lack of plant-to-plant transmission and the great reduction of in vitro aphid transmission of PVY 1-2. The first, a G to A change at nucleotide position 266, led to an Arg (PVY 0 AT) to Gln (PVY 1-2) change at amino acid position 89; and the second, a G to A mutation at nucleotide position 456, replaced a Met (PVY 0 AT) with Ile (PVY 1-2) at amino acid position 152 (Fig. 2). Interestingly, neither of these two PVY 1-2-specific amino acid changes at positions 89 and 152 occurred in the conserved HC domains involved in aphid transmission (KITC and PTK), which are present in most known potyviruses (Fig. 4). However, these two amino acids are highly conserved among PVY strains (Fig. 3). As reported for the transmissible PVY 0 AT (8), the amino acid sequence of PVY 1-2 HC has a KMTC motif instead of the highly conserved KITC motif. This motif, as well as the PTK motif, is suggested to be involved in aphid- and CP-binding, respectively (5,21).

DISCUSSION

A PVY isolate belonging to the pepper pathotype 1-2 was characterized as non-aphid transmissible in plant-to-plant transmission tests. Its loss of transmissibility may presumably be the result of successive mechanical inoculations by which mutations deleterious to aphid transmission could be incorporated into the virus genome, as has been found for other potyviruses (15,16,27). However, high rates of virus transmission of this isolate could be achieved if aphids were allowed to acquire biologically active HC prior to the virions in membrane tests. This indicated that viral CP was functionally active for transmission and suggested that a deficiency in HC was responsible for the efficiency of virus transmis-

Fig. 3. Alignment of two regions of helper component sequence of six potato virus Y (PVY) isolates and potyviruses tobacco vein mottle virus (TVMV), tobacco etch virus (TEV), plum pox virus (PPV), and turnip mosaic virus (TuMV). Arrows indicate the amino acid changes found between PVY 1-2 and PVY 0 AT. Amino acid differences are indicated below the PVY 1-2 sequence. Dots indicate the same residues are present. Conserved amino acids are shown in bold below the alignment. **A**, Alignment of amino acids 82 to 100. **B**, Alignment of amino acids 145 to 163.

Fig. 4. Alignment of two helper component regions containing highly conserved domains involved in aphid transmission of potato virus Y (PVY) isolates and potyviruses tobacco vein mottle virus (TVMV), tobacco etch virus (TEV), zucchini yellow mosaic virus (ZYMV), plum pox virus (PPV), and turnip mosaic virus (TuMV). Amino acid differences are indicated below the PVY 1-2 HC sequence. Dots indicate the same residues are present. Amino acids required for aphid transmission are boxed. **A**, Alignment of amino acids 34 to 56. **B**, Alignment of amino acids 302 to 316.

sibility. Surprisingly, PVY 1-2 HC preparations assisted low levels of aphid transmission of purified virions from PVY 0 and PVY 1-2 isolates. It is not clear why PVY 1-2 was weakly transmissible when aphids acquired purified virions mixed with PVY 1-2 HC through membrane, but was non-aphid transmissible in plant-to-plant assays. This phenomenon may be due to the presence of a higher concentration of HC, virions, or putative host factors associated with HC in the artificial feeding solution, as has recently been proposed (11).

Amino acid sequence analysis of the PVY 1-2 HC protein revealed two amino acid changes with respect to that of the aphid-transmissible PVY 0 AT (Gln to Arg, at position 89, and Ile to Met, at position 152) that affect amino acid residues highly conserved among PVY strains. Such mutations could be somehow involved in the aphid-transmission efficiency of PVY 1-2 by altering either the dimerization of the HC monomer (3,29) or the folding of the HC protein, masking any of the motifs required for HC-virion or HC-stylet interactions. However, we have shown that neither of the mutations found in the PVY 1-2 HC had a negative effect on its ability to bind virions; therefore, a plausible explanation for the loss of HC function is that either of these mutations, or both, render the HC incapable of interacting with the stylets. On the other hand, PVY 1-2 HC mutations might affect the stability of the protein in the host cell and, therefore, reduce the amount of available biologically active HC in the infected tissue in such a way that it is not sufficient to promote aphid transmission from plants.

It is important to note that the substitution of the basic Arg for the uncharged Gln at position 89 in the PVY 1-2 HC amino acids sequence lies in position -1 with respect to a conserved Phe (Fig. 3), suggesting that this change of charge might affect the ability of PVY 1-2 HC to mediate aphid transmission. In fact, a number of examples of loss of aphid transmission due to changes in the charge or polarity of a single amino acid in conserved domains of the HC sequence of potyviruses have been reported. A Lys to Glu change in the highly conserved KITC motif of the HC of TVMV (4), TEV (5), and potato virus C (PVC; 30) resulted in loss of HC activity. A substitution of Lys to Asn in the KMTC motif of PVY 0 (8) and Lys to Glu in the KLSC motif of ZYMV (13) have similar phenotypic effects. The presence of a positively charged amino acid at the first position of these conserved motifs, therefore seems to be essential for HC activity (4). Other mutations leading to charge changes in different regions of the HC adversely affecting HC activity have also been described. These include a Gly to Asp at position 35 of PVY 1 HC (8) and a Gly to Glu preceding the KITC sequence in TuMV (20).

The second amino acid replacement found in the PVY 1-2 HC at position 152 also occurred in position -1 with respect to a conserved Ala (Fig. 3). However, this mutation did not cause a charge change because both residues are hydrophobic nonpolar amino acids. Nevertheless, it would be not surprising if this change of Met to Ile were detrimental to aphid transmissibility of the PVY 1-2, because substitutions between amino acids with similar characteristics have been proved to abolish aphid transmission. Replacing Lys by His in the KITC motif of TVMV (4) resulted in loss of HC activity.

The mentioned mutations in highly conserved regions at the N terminus of the HC sequence of potyviruses are located within or near the cysteine-rich domain. Such motifs have been reported to form tertiary structures having functional roles and also may bind metal ions that could be required for HC biological activity (3, 23,26).

Recently, the loss of aphid transmissibility of TEV has been shown to be due to a substitution of Phe to Leu (both hydrophobic nonpolar amino acids) at nonconserved amino acid 10 of the TEV HC (5). Therefore, the PVY 1-2 HC mutations, which are located in nonconserved positions outside the cysteine-rich domain, also may be associated with the inability of HC to mediate highly efficient aphid transmission of PVY 1-2. Further information about the

spatial structure or folding of potyvirus HC is needed for a better understanding of the involvement in transmission of amino acid changes in the HC sequence.

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