Mutations in the helper component protease gene of zucchini yellow mosaic virus affect its ability to mediate aphid transmissibility

Herve Huet,1 Amit Gal-On,1 Efrat Meir,1 Herve Lecoq2 and Benjamin Raccah1*

1 Department of Virology, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet-Dagan 50-250, Israel and 2 Pathologie Vegetale, INRA, Montfavet, France

The nucleotide sequence of the helper component protease (HC-Pro) genes of three zucchini yellow mosaic virus (ZYMV) strains has been compared with that of a helper-deficient strain of ZYMV-HC. The comparisons revealed three unique deduced amino acid differences. Two of these mutations were located in regions which are conserved in other potyviruses. The role of these mutations in aphid transmissibility was examined by exchanging DNA fragments of part of the deficient HC-Pro gene with the respective section within the gene of the infectious full-length clone of the aphid-transmissible ZYMV. The first exchange included two of the three mutations, the first coding for a change from Asp to Gly (in a non-conserved region) and the second coding for a change from Arg to Ile [within the Phe-Arg-Asp-Lys (FRNK) conserved box]. This exchange resulted in a reduced transmission (20.6% for the mutated virus compared with 57.4% in the normal ZYMV when acquired from plants and 37.2% compared with 83.1%, respectively, when acquired from membranes). The second exchange incorporated a single mutation [confering a change from Thr to Ala within the Pro-Thr-Lys (PTK) conserved box]. This single mutation resulted in almost total loss of HC activity in aphid transmission both from plants and from membranes. The Lys residue in the conserved Lys-Ile-Thr-Cys (KITC) box, which is related to loss of HC activity in potato virus Y, tobacco vein mottling virus and in the Michigan strain of ZYMV, is unchanged in the helper-deficient ZYMV. It is therefore proposed that more than one site in HC-Pro may be functionally related to aphid transmissibility. The possible reasons for the role of these mutations in helper activity in aphid transmission of ZYMV are discussed.

Introduction

Transmission of potyviruses by aphids is dependent on a biologically active helper component (HC) and coat protein (CP) (Pirone, 1991). In most cases, lack of aphid transmissibility has been attributed to mutations in the amino-terminal region of CP (Atreya et al., 1990, 1991; Gal-On et al., 1992). Fewer reports are available for potyviruses lacking helper activity. Potato virus C (PVC), which lacks the ability to be transmitted by aphids (Bawden & Kassanis, 1947), was the first potyvirus found to have a deficient HC (Kassanis & Govier, 1971a, b). PVC is a helper-defective strain of potato virus Y (PVY), which produces an HC which is identical in size and in immunological reaction to PVY HC, but is incapable of assisting in aphid transmission of purified virions (Thornbury et al., 1990). Comparison of the predicted amino acid sequences of the HC-Pro genes of PVC, PVY and several other potyviruses revealed two amino acid changes within areas of conserved boxes, one from Lys to Glu [within the Lys-Ile-Thr-Cys (KITC) box and the second from Ile to Val [within the Ile-Asp (ID) box] (Thornbury et al., 1990). This mutation was also found in helper-deficient strains of zucchini yellow mosaic virus (ZYMV) from Michigan, U.S.A. (Grumet et al., 1992) and from Reunion Island (Granier et al., 1993). Later, when the Lys to Glu mutation was introduced into the KITC box of tobacco vein mottling virus (TVMV) HC-Pro, a total loss of helper activity was recorded (along with reduced virus accumulation and strong attenuation of symptoms) (Atreya et al., 1992).

ZYMV (Lisa et al., 1981) is one of the most important viruses in cucurbits worldwide. Lecoq et al. (1991b) have described a mild and very poorly aphid-transmissible ZYMV strain (ZYMV-WK) which is derived from a severe and helper-deficient isolate (ZYMV-PAT) (Lecoq et al., 1991a). Similarly to PVC, the helper-deficient ZYMV may be transmitted efficiently by aphids if coinfected with another potyvirus (Lecoq et al., 1991b), or if its virions are mixed with an HC deriving from another isolate of ZYMV (Lecoq et al., 1991a). In the present study, the HC-Pro gene of a helper-deficient
strain of ZYMV was compared with those of three normal strains of the virus, and with other potyviruses. The role of specific mutations within conserved regions of the HC-Pro gene in aphid transmissibility was investigated and the biological significance of the mutations discussed.

Fig. 1. Schematic representation of the strategy for constructing a full-length clone cDNA of ZYMV-HC(+) comprising sections of the HC-Pro gene of the helper-deficient strain ZYMV-HC(-). (a) Top bar, pZYHC(+) full-length clone has served to generate in vitro infective transcripts. Its non-coding region (NCR) at the 5' end is attached to the T7 promoter and at the 3' end, the poly(A) is tail-limited by the KpnI restriction site. The line with arrowheads designates the location at the BamHI site of the full-length clone where the subclones were ligated to the rest of the full-length clone. (A) pZYHC(+)-5' end is a subclone of pZYHC(+) full-length clone. It represents the 5' end of the genome up to the BamHI restriction site close to the 3' end of the HC-Pro gene. (B) pZYHC(-) is a cDNA fragment representing the HC-Pro gene of ZYMV-HC(-) (open bar); numbers at the left and right edges of the bar designate the position of the first nucleotide of the primers used for PCR cloning. Fragment exchanges resulted in two hybrid subclones, pZYHC(GI-T)-5' end (C) and pZYHC(DR-A)-5' end (D). Construction of the hybrid pZYHC(GI-T)-5' end (the GI-T designation stands for the amino acid change in the resulting HC-Pro). This clone was obtained by incorporating the 5' end of the HC-Pro gene of ZYMV-HC(-) (between AgeI and BstXI) in the pZYHC(GI-T)-5' end (C). Construction of the hybrid pZYHC(DR-A)-5' end (the DR-A designation stands for the amino acid change in the resulting HC-Pro). This clone was obtained by incorporating the 3' end of the HC-Pro gene of ZYMV-HC(-) (between BstXI and BamHI) in pZYHC(GI-T)-5' end. The resulting hybrid clones were ligated to the rest of the ZYMV(+ ) clone (see location in the top bar). (b) Comparison of the HC-Pro genes of ZYMV-HC(+) (top) and ZYMV-HC(-) (bottom). Numbers above the bar indicate nucleotide positions in relation to the putative 5' end of the gene [based on comparison with the cleavage site suggested for TVMV by Mavankal & Rhoads (1991)]. The restriction sites indicated above the bar are those used for construction of the hybrid HC-Pro genes. Numbers above the HC-Pro gene of ZYMV-HC(-) represent the positions of two specific restriction sites (DraI and MspI) which were used to identify the hybrid full-length clones. Numbers beneath the bar indicate amino acid positions in relation to the putative N terminus of the HC-Pro (Y/S cleavage site). A, Ala; I, Ile; R, Arg; T, Thr; D, Asp; G, Gly; Y, Tyr; S, Ser.
Methods

**Virus strains.** Two strains of ZYMV differing in HC-Pro were used in the present study. (i) A strain that is non-aphid-transmissible owing to coat protein deficiency, designated ZYMV-NAT, has a biologically active HC-Pro (Antignus et al., 1989; Bourdin & Lecoq, 1991; Lecoq et al., 1991a). ZYMV-NAT has been used to prepare a full-length clone, and infectious in vitro transcripts (Gal-On et al., 1991); it was rendered aphid-transmissible by introducing a mutation in the CP gene and has therefore been named ZYMV-NAT* (Gal-On et al., 1992). Here the ZYMV-NAT* strain will be referred to as ZYMV-HC(+) (indicating an active HC). (ii) ZYMV-WK is a strain that causes mild symptoms (Lecoq et al., 1991b). The ZYMV isolates from France produce a helper-deficient HC-Pro which assists transmission at a very low rate [hence designated ZYMV-PAT, for being poorly aphid-transmissible (Lecoq et al., 1991a)]. It is designated here ZYMV-HC(-) (indicating helper deficiency). The amino acid sequences of the HC-Pro of two additional ZYMV strains were included in the comparison, namely those of ZYMV-Cal (Balint et al., 1989) and ZYMV-Reu (Baker et al., 1992).

**Clones.** Single-stranded cDNA representing the 5' end of the ZYMV-HC(-) RNA was obtained by reverse transcription using a specific primer representing a conserved region which coincides with the putative 3' end of the HC-Pro gene starting at position 1367 from its putative 5' end (5'CACACTCTGAATGTTTCA3'). The single-stranded cDNA was then amplified using two primers, the one mentioned above and another which corresponds to a region which is located 78 nucleotides (nt) upstream from the 5' putative end of HC-Pro gene (5'GTGATTCCGAGGTAGAGACG3'). The DNA fragment produced by PCR was inserted in an EcoRV-digested pBluescript vector which was named pZYHC(-) (Fig. 1a, B). The authenticity of the PCR product was inserted in a pBluescript vector which was named pZYHC(-) (Fig. 1a, B) at the respective site in pZYHC(+) resulted in subclone pZYHC(DR-A)-5' end containing the C terminus of HC-Pro (including mutation A instead of T) (Fig. 1a, C). The exchange was confirmed by the presence of the BamHI restriction site (Fig. 1b, bottom).

In vitro transcription. In vitro transcription was carried out as described by Gal-On et al. (1992). One-hundred ng/μl of KpnI-linearized full-length clone was transcribed using 20 units/μl of T7 RNA polymerase (Stratagene) and 1 unit/μl of RNasin (Promega) and 1 mM of mgPPpG (New England Biolabs).

**Plant inoculation.** The procedure was as described in Gal-On et al. (1992). In vitro capped RNA transcripts were diluted 1:1 with distilled water and applied to 8-day-old squash or cucumber seedlings. The cotyledon surface of squash was dusted with carborundum and mechanically infected with 30 μl of the diluted transcription mixture. The progeny viruses of these seedlings were used for mechanical inoculation of plants to be tested for HC purification and transmission.

**Determination of the mutations in the progeny viruses.** To ascertain the presence of the mutations in the viral RNA, total mRNA from infected leaf tissue was isolated, and cDNA to the HC-Pro-specific RNA was amplified by PCR and then analysed for the presence of specific restriction sites. mRNA extraction was carried out using Dynabeads in an mRNA purification kit (Dynal). The first cDNA strand was synthesized in a 20 μl volume containing 50 ng of total mRNA, 10 units/μl avian myeloblastosis virus reverse transcriptase (Molecular Genetic Research) and 300 ng of the above-mentioned primer for the 3' end of the HC-Pro gene. Then 2 μl of this mixture was used for PCR, in a 50 μl volume containing 300 ng of two primers, the 3' one and another (see above) which is specific to a region in the 5' end of the HC-Pro gene. The DNA fragment was then digested by DraI and MspI.

Simultaneous inoculation of squash seedlings with wild-type and mutated virus strains. Squash seedlings were inoculated simultaneously and with the same amount of virus-infected leaf. The two ancestor virus strains [ZYMV-HC(+)] and ZYMV-HC(-)] and the two mutated strains [ZYMV-HC(5'T) and ZYMV-HC(3'5'T)][, which were derived from the hybrid full-length clones, were used. Mock-inoculations were included as a control.

The mock-inoculated plants and those infected with the ancestors and mutant ZYMV strains served for comparison of virus titre for each of the strains, by ELISA, and for the determination of helper activity in aphid transmission of partially purified HC. These tests were carried out 3 weeks after inoculation.

**Determination by ELISA of ZYMV-CP titre in plants.** Lacking a specific and potent antibody against the ZYMV HC, we compared the CP titre in plants infected with the four strains, assuming that the CP titre reflects the titre of the HC processed from the same polyproteins. Discs of approximately 25 mg were taken from each of the infected plants which were used for HC purification. The discs were homogenized in 500 μl of PBS-Tween 3 weeks after inoculation (Table 1). Virus titres were compared by ELISA using a rabbit anti-ZYMV
serum. The ELISA procedure and antisera were those used by Antignus et al. (1989).

Helper purification and membrane aphid transmission. The squash seedlings inoculated simultaneously as described above were used for HC purification. Three weeks after inoculation, 10 g of leaves was harvested and served for HC purification from plants which had been inoculated with each of the four virus strains or from the control. Helper purification was as described by Antignus (1989). Comparison was also made with three strains of ZYMV which have highly active HC: ZYMV-Cal from California (Balint et al., 1990), ZYMV-Reu from Reunion (Baker et al., 1992) and ZYMV-HC(+) from Bet Dagan (A. Gal-On, N. Chejanovsky, H. Huet & B. Raccah, unpublished). Comparison was also made with the deduced amino acid sequence of HC-Pro of several additional potyviruses (Fig. 2). The comparison revealed three mutations unique to ZYMV-HC(−). One of the mutations (Asp to Gly) is in a non-conserved region (at position 148 from the putative N terminus of HC-Pro)

### Table 1. Comparison of ZYMV-CP titre by ELISA for plants which were infected with wild-type and mutated HC-Pro gene of ZYMV strains*

<table>
<thead>
<tr>
<th>Virus strain†</th>
<th>Expt 1</th>
<th>Expt 2</th>
<th>Expt 3</th>
<th>Expt 4</th>
<th>Pooled average</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYMV-HC(+)</td>
<td>1.11 ± 0.19 9§</td>
<td>0.69 ± 0.30 8</td>
<td>1.18 ± 0.08 8</td>
<td>1.26 ± 0.10 8</td>
<td>1.17 ± 0.27 8</td>
</tr>
<tr>
<td>ZYMV-HC(−)</td>
<td>1.05 ± 0.33 3</td>
<td>1.01 ± 0.23 8</td>
<td>1.28 ± 0.22 8</td>
<td>1.28 ± 0.15 8</td>
<td>1.09 ± 0.28 8</td>
</tr>
<tr>
<td>ZYMV-HC(GI-T)</td>
<td>0.67 ± 0.29 9</td>
<td>0.80 ± 0.21 9</td>
<td>0.81 ± 0.19 9</td>
<td>0.90 ± 0.04 9</td>
<td>0.80 ± 0.22 9</td>
</tr>
<tr>
<td>ZYMV-HC(DR-A)</td>
<td>0.82 ± 0.15 9</td>
<td>0.99 ± 0.09 9</td>
<td>1.09 ± 0.22 9</td>
<td>1.25 ± 0.09 9</td>
<td>1.04 ± 0.21 9</td>
</tr>
</tbody>
</table>

* Five squash plants for each strain were inoculated mechanically with the same amount (g/ml) of infected leaves in each experiment. Samples of 25 mg were taken from each plant 3 weeks after inoculation.
† ZYMV-HC(+) and ZYMV-HC are described in the text. ZYMV-HC(GI-T), mutated strain where D was replaced by G in a non-conserved region and R by I in the FRNK conserved box. ZYMV-HC(DR-A), mutated strain where T was replaced by A in the PTK conserved box.
‡ ELISA values were determined using rabbit anti-ZYMV-CP antibodies.
§ Within a strain, mean values ± s.d. followed by a common letter are not significantly different (P < 0.01; Student’s t-test).

Fig. 2. Comparison of conserved regions in HC-Pro which were found to differ in the helper-deficient strain of ZYMV from that of other potyviruses. The numbers indicate the location of the amino acids in respect to the putative N terminus of HC-Pro. Solid and dashed boxes designate conserved or non-conserved regions (respectively). Asterisks indicate mutations in the helper-deficient strain of ZYMV. Bold letters designate amino acids which are present in conserved regions in other potyviruses. PPV, plum pox virus; TEV, tobacco etch virus; TuMV, turnip mosaic potyvirus; PRSV, papaya ringspot virus; PSbMV, pea seed-borne mosaic virus.

**Results**

Comparison of the HC-Pro gene of the helper-deficient ZYMV-HC(−) with that of other ZYMV strains and other potyviruses

The nucleotide sequence and the computer-derived deduced amino acid sequence of the HC-pro gene of ZYMV-HC(−) have been sent to the EMBL Data Library. A comparison was made with three strains of ZYMV which have highly active HC: ZYMV-Cal from California (Balint et al., 1990), ZYMV-Reu from Reunion (Baker et al., 1992) and ZYMV-HC(+) from Bet Dagan (A. Gal-On, N. Chejanovsky, H. Huet & B. Raccah, unpublished). Comparison was also made with the deduced amino acid sequence of HC-Pro of several additional potyviruses (Fig. 2). The comparison revealed three mutations unique to ZYMV-HC(−). One of the mutations (Asp to Gly) is in a non-conserved region (at position 148 from the putative N terminus of HC-Pro)
ZYMV HC-Pro and aphid transmissibility

Table 2. Transmission of ZYMV from plants infected with the wild-type ZYMV-HC(+), ZYMV-HC(−) and the mutated ZYMV-HC(GI-T) and ZYMV-HC(DR-A)*

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Aphids per test plant</th>
<th>Rate of infection (infected/tested)</th>
<th>Expt 1</th>
<th>Expt 2</th>
<th>Expt 3</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYMV-HC(+)</td>
<td>4</td>
<td>9/16</td>
<td>10/17</td>
<td>8/18</td>
<td>27/47</td>
<td>57.4</td>
</tr>
<tr>
<td>ZYMV-HC(−)</td>
<td>4</td>
<td>0/21</td>
<td>10/19</td>
<td>0/20</td>
<td>0/60</td>
<td>0</td>
</tr>
<tr>
<td>ZYMV-HC(GI-T)†</td>
<td>20</td>
<td>0/37</td>
<td>0/21</td>
<td>0/20</td>
<td>0/78</td>
<td>0</td>
</tr>
<tr>
<td>ZYMV-HC(DR-A)</td>
<td>4</td>
<td>0/16</td>
<td>0/17</td>
<td>0/15</td>
<td>0/48</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0/20</td>
<td>0/21</td>
<td>0/20</td>
<td>0/61 0</td>
</tr>
</tbody>
</table>

* Acquisition access feeding for 5 min. Inoculation access feeding for 18 h.
† The transmissibility of ZYMV-HC(GI-T) was retested from test plants which were infected in replicate no. 1 and the rate of transmission was 13/67 (19.4%).

Table 3. Membrane transmission of ZYMV-AT virions mixed with HCs deriving from the wild-type and mutated ZYMV strains

<table>
<thead>
<tr>
<th>Virus strain*</th>
<th>Rate of infection† (infected/tested)</th>
<th>Expt 1</th>
<th>Expt 2</th>
<th>Expt 3</th>
<th>Expt 4</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYMV-HC(+)</td>
<td>14/17†</td>
<td>13/17†</td>
<td>15/18†</td>
<td>17/19†</td>
<td>59/71</td>
<td>83.1</td>
</tr>
<tr>
<td>ZYMV-HC(−)</td>
<td>0/21 e</td>
<td>0/17 e</td>
<td>1/25†</td>
<td>0/19†</td>
<td>1/82</td>
<td>1.2</td>
</tr>
<tr>
<td>ZYMV-HC(GI-T)</td>
<td>8/33 h</td>
<td>14/29 h</td>
<td>9/20 h</td>
<td>7/20 h</td>
<td>38/102</td>
<td>37.2</td>
</tr>
<tr>
<td>ZYMV-HC(DR-A)</td>
<td>0/21 e</td>
<td>1/29 e</td>
<td>0/17 e</td>
<td>0/33 e</td>
<td>1/100</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* See Table 1 footnote.
† Acquisition from a mixture of partially purified HC and 100 g purified ZYMV-AT virions in 0.2 M-Tris-sulphate buffer pH 7.2 containing 0.02 M-MgCl₂ and 20% sucrose. Acquisition access feeding for 10 min, inoculation access feeding for 18 h. Ten aphids which completed a 10 min acquisition access feeding were placed on each test plant.
‡ Within a strain, mean values ± s.d. followed by a common letter are not significantly different (P < 0.05; χ² test).

(Fig. 2). The mutations in conserved boxes were from Arg to Ile (within the FRNK box) and from Thr to Ala (within the PTK box) at positions 180 and 309 from the putative N terminus (Fig. 2).

The Lys residue in the conserved KITC box that was related to helper deficiency in PVC (Thornbury et al., 1990) was also found in helper-deficient ZYMV strains (Grumet et al., 1992; Granier et al., 1993). This mutation, when introduced into the HC-Pro gene of TVMV, caused loss of helper activity (Atreya et al., 1992). The KITC box in the HC-Pro gene of the helper-deficient ZYMV-HC(−) is identical to that of three other ZYMV strains and to that of soybean mosaic virus (SMV; Jayaram et al., 1992), showing a consistent variation of Ile to Leu and Thr to Ser (Fig. 2).

Identification of the mutations that affect helper activity in ZYMV

Two mutations were incorporated into the 5’ end of the ZYMV-HC(+)-HC-Pro gene, which resulted in two amino acid changes in the N terminus of HC-Pro (Asp to Gly and Arg to Ile). This was achieved by introducing a cDNA fragment (from AgeI to BstXI) from the HC-Pro gene of ZYMV-HC(−) into subclone pZYHC(+)-5’ end (Fig. 1a), after which the subclone was ligated to another subclone representing the remainder of ZYMV-HC(+) to form the hybrid pZYHC(GI-T) full-length clone (Fig. 1a, C). Plants infected with transcripts from the latter clone served for aphid transmission (Table 2).

As seen in Table 2, transmissibility of virus from plants was reduced from the average transmission rate of 57.4% for those inoculated with the original ZYMV-HC(+), to 20.6% for those infected with ZYMV-HC(GI-T). The activity of partially purified HC extracted from plants infected with ZYMV-HC(+) or with ZYMV-HC(GI-T) was also tested by comparing transmission of virus acquired from membranes. As shown in Table 3, transmission was reduced from the average transmission rate of 83.1% for the active ZYMV-HC(+) HC to 37.2% for HC produced by the hybrid ZYMV-HC(GI-T). This reduction in aphid transmission could result from an effect of the mutation either on HC ability to mediate transmission, or on the titre of HC in the plant. A certain reduction in virus titre for ZYMV-HC(GI-T) is seen in Table 1.
The third mutation (Thr to Ala) was present in the 3′ half of the HC-Pro gene (Fig. 1b). A similar procedure was repeated exchanging the respective fragment (from BstXI to BamHI) from ZYMV-HC(−), first into the subclone pZYHC(+)-5′ end and then the subclone was ligated to the remainder of the genome to form the hybrid pZYHC(DR-A) full-length clone (Fig. 1a, D). The transcripts obtained were then used to inoculate plants which served for transmission of ZYMV (Table 2). Attempts to transmit the ZYMV-HC(DR-A) by aphids have failed even when the number of vectors was increased by placing 20 aphids instead of four on each test plant (Table 2). Average aphid transmission rates for partially purified HC acquired from membranes was similar for ZYMV-HC(DR-A) and ZYMV-HC(−) HCs (1-0% and 1-2% respectively) and much reduced compared with the rate recorded for the original HC of ZYMV-HC(+) (83-1%) (Table 3). Unlike the former mutations, HC produced by ZYMV-HC(DR-A) failed almost completely to mediate aphid transmission. Virus CP titre, as determined by ELISA, was almost the same in ZYMV-HC(DR-A) as that found in ZYMV-HC(+) (Table 1).

Discussion

Possible mechanisms for the helper-mediated transmission of potyviruses by aphids were discussed by Pirone & Thornbury (1984), by Murant et al. (1988) and updated by Pirone (1991). The most likely explanation for the role of the helper is in binding the virus to the aphid’s mouthparts (Berger & Pirone, 1986). However, direct evidence for such a function is still lacking. Other hypotheses are that HC facilitates release of virus from the stylet or protects the virus from adverse conditions in the aphid’s alimentary tract (Pirone & Thornbury, 1984).

Comparison between the HC-Pro gene of PVC and those of two other PVY strains and three other potyviruses was made by Thornbury et al. (1990). Since then, additional HC-Pro sequences of potyviruses have become available and the N terminus of the HC-Pro gene of TVMV has been determined (Mavankal & Rhoads, 1991). This allowed comparison of the amino acid sequence of the helper-deficient ZYMV-HC(−) HC-Pro with that of three other ZYMV strains, and also with several other potyviruses. The comparison revealed three mutations which were found only in ZYMV-HC(−), two of which were within regions that are conserved in almost all other potyviruses. Neither of the two mutations is located within the protease part of the HC-Pro. The two helper-deficient potyviruses, PVC (Thornbury et al., 1990) and ZYMV-HC(−), differed in the mutations that were responsible for loss of activity. They also differ biologically: PVC has a totally inactive HC (Pirone, 1991) whereas ZYMV-HC(−) occasionally assists virus transmission at a very low rate (Lecoq et al., 1991a). Lacking an infectious full-length clone for PVY or PVC, Atreya et al. (1992) used the full-length clone of TVMV to demonstrate the role of the KITC box in aphid transmission by introducing several mutations in its HC-Pro gene. Replacement of Lys with Glu in the TVMV HC-Pro was found to confer loss of helper activity (Atreya et al., 1992).

In ZYMV, the KITC conserved box appears in the KLSC form. That Lys is unchanged in the KLSC box in the helper-deficient strain of ZYMV-HC(−) suggests that more than one region is involved in helper activity. This does not suggest a unique form of deficient HC-Pro, as Grumet et al. (1992) and Granier et al. (1993) reported a mutation in Lys in this conserved box. The KITC box form is common in most potyviruses. The change from Ile and Thr in the KITC to Leu and Ser was found also in SMV (Jayaram et al., 1992), probably representing a variation of the conserved box within potyviruses.

The partial reduction of HC activity recorded when the N terminus of the HC-Pro was replaced can be attributed either to a change in the ability of HC to mediate transmission or to the lower titre of the virus, and therefore of HC, in the infected plants. Lacking a specific antibody against ZYMV-HC, direct serological visualization of HC titre in plants and partially purified preparations was not possible, and therefore the exact reason for the reduced activity caused by these mutations remains unknown.

The replacement of the C terminus of the HC-Pro in ZYMV-HC(+), which introduced a single change of amino acid (Thr to Ala), resulted in a helper activity as poor as that recorded for ZYMV-HC(−). This suggests a major role of the PTK conserved box in helper activity in aphid transmission.

Unlike the mutation in the KITC box of TVMV, which affected virus accumulation (Atreya et al., 1992), the mutation in both the FRNK and the PTK boxes of ZYMV had a slight effect on virus titre.

Contrary to the situation for the potyvirus CP, for which a general organization has been proposed (Shukla & Ward, 1989), little is known about the three-dimensional structure of the HC in potyviruses. There is a possibility that either the KITC and/or the PTK conserved regions are involved in protein folding, thus exposing regions which are essential for aphid transmission. However, some evidence has been provided that it is biologically active in a dimer form (Thornbury et al., 1985). Another attempt to speculate about the function of HC was made in view of the cysteine-rich regions which were reported in the HC of PVY (Robaglia et al., 1989). Cysteine-rich regions are known to assume zinc-finger functions such as protein–nucleic acid binding.
and/or in folding of proteins (Semmens & Jeang, 1992). That the mutation in the KITC box falls within a cysteine-rich region (Thornbury et al., 1990) led Atreya et al. (1992) to ascribe a possible binding function to HC. Lacking direct proof of the mode of action of these mutations in aphid transmission, two possible mechanisms are suggested here. (i) HC serves as a link between the virus and the stylet. In this case, two amino acids are required for binding; thus one box (either KITC or PTK) is required to attach to the virus and the other (either KITC or PTK) is required to attach to the aphid’s stylet. (ii) HC is biologically active only in a dimer form. The KITC and the PTK boxes are co-involved in dimer formation of the biologically active helper. A mutation in either will therefore interfere in dimer formation and will abolish activity.

Further mutations in TVMV and in ZYMV as well as in other potyviral systems are required to shed additional light and provide additional knowledge on the mode of action of HC in aphid transmission.

The authors are grateful to Mrs Sima Singer for excellent technical assistance, to Drs E. Hiebert and C. Baker for prepublished information and to Drs Victor Gaba and Y. Antignus for critical reading of the manuscript. This research was supported in part by a grant from the BARD no. US-1866-1990. Contribution No. 1066-E, from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

References


Huet, H. & others


(Received 10 August 1993: Accepted 4 January 1994)