Mutations in zucchini yellow mosaic virus helper component protein associated with loss of aphid transmissibility

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Zucchini yellow mosaic virus (ZYMV) is a potyvirus transmitted by aphids in a non-persistent manner. Isolates having partially or totally lost their ability to be transmitted by aphids have been isolated and found to be affected in their helper component activities. We have sequenced the helper component coding region of poorly aphid-transmissible (PAT) variants of two strains of ZYMV, E15 and R5A. Mutations have been identified at the nucleotide level leading to two amino acid changes in the E15 PAT variant helper component and to one amino acid change located in the cysteine-rich region (well-conserved among potyviruses) in R5A PAT variant helper component. The mutation in the R5A variant changes the same amino acid as the one identified in potato virus C, a non-transmissible strain of potato virus Y.

Zucchini yellow mosaic virus (ZYMV) (Lisa et al., 1981) is a widely distributed potyvirus that causes important losses in a range of cucurbit crops (Lisa & Lecoq, 1984). ZYMV is very efficiently transmitted from plant to plant by several aphid species in a non-persistent manner (Lisa & Lecoq, 1984).

Two viral proteins are involved in aphid transmission of potyviruses: the coat protein (CP) and the helper component (HC) (Pirone, 1991). The size of the biologically active HC form is 106K for tobacco vein mottling virus (TVMV) and 116K for potato virus Y (PVY) (Hellman et al., 1983). These active forms can be resolved as 53K (TVMV) or 58K (PVY) proteins by denaturing gel electrophoresis, suggesting that the protein is a homodimer (Thornbury et al., 1985). The monomeric HC protein is cleaved from the viral polyprotein by two proteolytic activities. The first one acts near the C-terminal part of the P1 protein and generates the N terminus of the HC (Mavankal & Rhoads, 1991; Verchot et al., 1991). The HC itself has a second proteolytic active site located on its 20K C-terminal part allowing its autocleavage for C terminus generation (Carrington et al., 1989a, b).

Very little information is available concerning the interactions between the virus particle, the HC and the aphid vector. Viruses showing differences in their transmissibility have been isolated after successive mechanical inoculations (Murant et al., 1988; T.

Table 1. Aphid transmission of two purified zucchini yellow mosaic virus strains in the presence of extracts containing homologous or heterologous HC

<table>
<thead>
<tr>
<th>Aphid feeding source*</th>
<th>Virus HC</th>
<th>Transmission†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYMV-R5A</td>
<td>ZYMV-R5A</td>
<td>29/30†</td>
</tr>
<tr>
<td>ZYMV-R5A-PAT</td>
<td>ZYMV-R5A-PAT</td>
<td>0/30</td>
</tr>
<tr>
<td>ZYMV-R5A-PAT</td>
<td>ZYMV-R5A</td>
<td>24/30§</td>
</tr>
<tr>
<td>ZYMV-R5A</td>
<td>ZYMV-R5A-PAT</td>
<td>0/30</td>
</tr>
<tr>
<td>ZYMV-R5A</td>
<td>ZYMV-R5A</td>
<td>0/30</td>
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<tr>
<td>ZYMV-R5A-PAT</td>
<td>ZYMV-R5A</td>
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<td>ZYMV-R5A-PAT</td>
<td>ZYMV-R5A-PAT</td>
<td>0/30</td>
</tr>
</tbody>
</table>

* Solutions contained a combination of purified virions (80 μg/ml), HC-containing extracts and 20% sucrose. Purified virus preparations and HC-containing extracts were obtained as described by Lecoq et al. (1991). When either HC or virus were omitted they were replaced by an equal volume of the respective buffer.
† M. persicae were allowed a 10 min acquisition access period before being transferred in groups of 10 to melon test plants.
§ Results are expressed as the number of plants infected divided by the number of plants inoculated (cumulated data from three independent experiments).

The nucleotide sequence reported in this paper will appear in the EMBL nucleotide sequence database under the accession number Z22759.
### Short communication

**Fig. 1.** Deduced amino acid sequences of HC of ZYMV strains E15 AT and PAT and R5A PAT. The sequence of R5A AT was from Baker *et al.* (1991). The PAT-specific amino acid differences are shown below the AT sequence. Amino acid differences between the two strains are in bold type on the R5A AT sequence. The region covering amino acids 281 to 320 of ZYMV-R5A AT was sequenced and called R5A AT*.
Legavre, personal communication) and shown to lack biologically active HC or to have changes in the CP.

Loss of transmissibility associated with CP has been reviewed by Atreya et al. (1990) and Gal-On et al. (1992) and was associated with modifications within a DAG amino acid triplet located next to the N-terminal part of the CP, which is conserved in all aphid-transmissible (AT) potyviruses (Atreya et al., 1991). An aphid non-transmissible isolate of ZYMV deficient in CP but capable of producing an active form of HC has been described (Antignus et al., 1989; Gal-On et al., 1992). It has a DTG triplet instead of the DAG.

Loss of transmissibility associated with HC deficiency was also reported (Kassanis & Govier, 1971; Thornbury et al., 1990; Lecoq et al., 1991) both for ZYMV isolates and potato virus C (PVC). Thornbury et al. (1990) identified two mutations in the HC coding sequence of PVC potentially leading to the loss of aphid transmissibility. Atreya et al. (1992) further demonstrated by using site-directed mutagenesis of an infectious TVMV clone that a lysine to glutamic acid change, previously identified in the PVC sequence and located within a conserved cysteine-rich domain (Robaglia et al., 1989), was responsible for the HC deficiency. Interestingly, this amino acid change was also found to affect symptom expression and virus accumulation.

A poorly aphid-transmissible (PAT) variant of ZYMV-E15 (designated ZYMV-E15-PAT), a strain originating from Southern France, was found to be deficient in the HC activity (Lecoq et al., 1991). In the course of the study of a ZYMV isolate (R5A), serologically distinct from the ZYMV type strain and originating from Reunion Island (Baker et al., 1991), a subculture that had lost aphid transmissibility was obtained following three single local lesion transfers on Chenopodium amaranticolor and C. quinoa. By analogy with ZYMV-E15-PAT, we will refer hereafter to this variant as ZYMV-R5A-PAT. In aphid transmission experiments, using three viruliferous Myzus persicae per test plant, ZYMV-R5A and ZYMV-R5A-PAT subcultures were transmitted to 71.6% and 0% of 60 melon plantlets, respectively. ZYMV-R5A-PAT was proved to be deficient in HC activity since it was transmitted when assisted by HC preparations from plants infected by ZYMV-R5A (Table 1). In this paper, we compared the nucleotide sequence of the HC regions of the AT form of ZYMV-E15 and its PAT variant. The nucleotide sequence of the PAT variant of ZYMV-R5A was compared with the sequence of the AT isolate established by Baker et al. (1991).

Virus purifications were done according to Lecoq & Pitrat (1985). Viral RNA was extracted according to Robaglia et al. (1989) or by treating resuspended virus with 1% SDS and 1 mg/ml proteinase K followed by phenol–chloroform extraction and ethanol precipitation. Purified RNA was reverse-transcribed using random hexanucleotide primers and Moloney murine leukemia virus reverse transcriptase. Double-stranded cDNA was cloned in Bluescript vectors (Stratagene). Single-stranded DNA was prepared with the helper phage M13K07. Nucleotide sequences were determined by the dideoxy-nucleotide chain termination procedure of Sanger et al. (1977) using an Applied Biosystems DNA sequencer 370A with either dye primers (Universal-21M13 and M13 reverse primers from Applied Biosystems) or dye terminators. Sequence data were analysed with the fragment assembly program of the Genetics Computer Group (Devereux et al., 1984).

Initial clones were identified by homology with published potyvirus sequences, then adjacent clones were isolated by colony hybridization. In some cases, sequence
determinations were done after PCR amplification and cloning of specific portions of the genome. Each nucleotide determination was done on at least two different clones.

The ZYMV HC N terminus was identified by amino acid sequence similarity with the cleavage site defined by Mavankal & Rhoads (1991) for TVMV and its C terminus by similarity with the G/G cleavage site defined by Carrington et al. (1989a) between HC and the P3 protein. The sequence covers 1368 nucleotides and 456 amino acids.

We found two nucleotide sequence differences between AT and PAT ZYMV-E15. An A to G change at position 443 led to an aspartic acid (PAT variant) change at position 148. This mutation can suppress a negative charge in the PAT variant HC protein. A second mutation, A to G at position 925, changes a threonine (AT variant) to an alanine (PAT variant) at position 309 (Fig. 1). The amino acid change occurring at position 148 lies in a region with no homology between members of the potyvirus family. This mutation lies in position +6 in respect to a conserved glycine and +4 in respect to a conserved alanine (Fig. 2a). By contrast, the second mutation (threonine to alanine at position 309) lies within a very conserved cluster of three amino acids (proline–threonine–lysine), itself localized in a domain supposed to be involved in the C-terminal proteolytic activity (Fig. 2b). We have moreover established, using oligonucleotides designed following the sequence of Grumet & Fang (1990) that the coat protein sequences of the E15-AT and PAT variants display a DAG triplet towards their N terminus confirming that the CP was not involved in the PAT variant phenotype.

For the PAT strain of the R5A isolate, the sequence of the cDNA clones was aligned with the published sequence of the ZYMV AT R5A (Baker et al., 1991). Four nucleotide sequence changes (A to G at position 154, A to G at position 267, C to G at position 912 and G to C at position 913) were found. Examination of the amino acid sequence of potyviruses in the region where amino acid mutations 304 (change at nucleotide 912) and 305 (change at nucleotide 913) were found reveals that most of them bear a glutamic acid–leucine doublet. We have therefore amplified and sequenced a fragment containing this region of the R5A-AT variant and found that the sequence was identical between the AT and PAT variants. The differences in sequence from the sequence obtained by Baker et al. (1991) thus have no influence on HC activity. The mutation occurring at position 267 does not change the amino acid sequence; by contrast, the mutation at position 154 leads to an amino acid change where the lysine 52 in the AT isolate is replaced by a glutamic acid in the PAT isolate (Fig. 1).

This is the same modification as that first noticed by Thornbury et al. (1990) between PVY and PVC and further confirmed by Atreya et al. (1992) using an infectious TVMV clone. Surprisingly, plants infected by the mutant TVMV-307 of Atreya et al. (1992) (in which the mutation changing a lysine to a glutamic acid at this
position has been introduced) were found to display delayed symptom development and a lower virus accumulation compared with wild-type AT virus. This was not the case for the PAT variant of ZYMV-R5A which multiplies as efficiently as the AT form, and induces severe symptoms (H. Lecoq, unpublished). Since this mutation is the only one leading to an amino acid change in the R5A-PAT strain it is conclusively responsible for the PAT phenotype. Grumet et al. (1992) identified three amino acid differences between the HC of a ZYMV PAT variant from Connecticut (CT) and HC of ZYMV AT strains, one of which is also the lysine to glutamic acid change. They however were not able to determine unambiguously which mutation was important for transmissibility.

The adenine to guanine transition leading to the lysine to glutamic acid change within the homologous cluster of amino acids lying between positions 24 and 60 (Fig. 3) has now been observed in three independently obtained potyvirus PAT isolates: in PVC (Thornbury et al., 1990), in a PAT isolate of PVY (T. Legavre, personal communication) and in ZYMV-R5A (this work). This opens the possibility that genomes bearing these mutations in the HC coding region were preferentially selected under conditions in which aphid transmission is not a constraint on survival.

Successful aphid transmission of potyviruses is a complex process, involving virus–HC–aphid interactions at multiple levels such as HC/CP recognition, interaction with aphid components, dimerization of HC monomers and interaction with unknown cofactors. Structural determinants as well as defined primary amino acid sequence determinants of the HC are probably required for these interactions. In this study we observed that at least two domains of the HC are involved in the decrease of aphid transmissibility. The availability of mutant proteins will help in further investigation of the factors affecting this process.

References


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