Analysis of potato virus X coat protein genes in relation to resistance conferred by the genes Nx, Nb and Rx1 of potato

Simon Santa Cruz*† and David Baulcombe

The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK

The coat protein gene nucleotide sequences from eight previously uncharacterized strains of potato virus X (PVX) were determined. Comparison of the deduced amino acid sequences showed that two classes of PVX coat protein, designated types X and B, could be distinguished based on protein length and overall amino acid identities. In all there were 14 amino acid positions where all of the type X proteins differed from all of the type B proteins. The PVX coat protein is the principal viral determinant of the outcome of interactions between the virus and potatoes carrying either the Nx or Rx1 resistance genes. The different strains of PVX were tested for their ability to overcome resistance conferred by three potato resistance genes: Nx, Nb and Rx1. All of the strains that were avirulent on potato cultivars carrying the Nx resistance gene were found to have type X coat proteins whereas strains capable of overcoming the Nx resistance had type B coat proteins.

Strains of potato virus X (PVX) can be classified into groups based on their ability to overcome resistance conferred by two hypersensitivity genes, Nx and Nb, found in potato (Solanum tuberosum L.). Group one strains induce a hypersensitive response (HR) in potatoes carrying either Nx or Nb, group two strains induce an HR only on Nb potatoes whereas group three strains only induce the Nx-mediated HR. Group four strains overcome both Nx- and Nb-mediated resistance (Cockerham, 1955). A further type of resistance to PVX, which is not associated with a hypersensitive response, is conferred by the genes Rx1 and Rx2, respectively (Ritter et al., 1991).

The genetics of viral pathogenicity and host resistance in the PVX–potato pathosystem can be viewed from the standpoint of the gene-for-gene model of plant–pathogen interactions (Flor, 1971; Keen, 1990). Thus the outcome of any interaction between PVX and potato is determined by the genetic constitution of both plant and virus. When resistance is conferred by both Nx and Rx1 it has been shown that the viral coat protein gene is the principal determinant of the host response (Kavanagh et al., 1992; Goulden et al., 1993; Santa Cruz & Baulcombe, 1993). The objective of this analysis was to assess the degree of variation between the coat proteins of naturally occurring strains of PVX and to correlate differences in the primary structure of the coat protein with variation in the outcome of interactions with potatoes carrying different PVX resistance genes.

To establish the pathotypes of the strains used in this analysis potato cultivars carrying different PVX resistance genes were inoculated with each virus. All virus strains were maintained by propagation on Nicotiana clevelandii. Each strain was inoculated into two plants of each of the following potato genotypes: nx nb rx (either Maris Bard or Desiree), Nx nb rx (either King Edward or Southesk), nx Nb rx (either Catriona or Arran Victory) and Rx1 (Cara). Inoculations were performed by grafting potato rootstock with scions of the tomato cultivar Ailsa Craig that had been manually inoculated with virus 2 weeks prior to grafting. Controls were performed by grafting healthy tomato scions to each of the potato cultivars. Following graft inoculations axillary shoots that developed from the potato rootstocks were monitored for the appearance of symptoms and 21 days after grafting leaf tissue was taken from potato leaflets for conducting an ELISA using PVX specific antibodies. ELISA was performed as described previously (Kavanagh et al., 1992) using the monoclonal antibody MAC58 (supplied by MAFF) that reacts with all tested strains of PVX (Torrance et al., 1986). For inoculations into Nx, Nb and Rx1 cultivars plants were considered susceptible when axillary shoots tested positive for PVX.

* Author for correspondence. Fax +44 1382 562426. e-mail virss@scri.sari.ac.uk

† Present address: Department of Virology, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.

The nucleotide sequence data reported in this paper have been deposited in the EMBL database and assigned the accession numbers X88781–X88788.
antigen and remained free of necrotic symptoms. For either \(Nx\) or \(Nb\) potato cultivars plants were considered resistant when the potato axillary shoots developed apical necrosis and tested positive for PVX antigen. For the \(Rx\) cultivar resistance was defined as the complete absence of PVX antigen in the potato axillary shoots.

The data presented in Table 1 show the strain groups for the different strains deduced from the phenotype of their interactions with differentiating potato cultivars. For the strains CP4, DX, DY, EX, HB, KP, UK3 and WS2 the results were consistent with those previously published (Kavanagh et al., 1992; Jones, 1982; Jones, 1985; Moreira et al., 1980; Torrance et al., 1986). For the strains that have not been described before, NL1 and XS belong to strain group one, XA belongs to strain group three and NL4 is a group four strain. With the exception of the previously characterized strain HB none of the strains included in this analysis was able to overcome resistance mediated by the \(Rx\) gene in the cultivar Cara.

The coat protein gene sequences of the PVX strains HB, UK3 (Kavanagh et al., 1992), CP4 (Goulden et al., 1993) and DX (Santa Cruz & Baulcombe, 1993) have been reported previously. The coat protein sequences of the other PVX strains included in this analysis were obtained from cDNA clones as described below. Total RNA prepared from \(N. clevelandii\) plants infected with each of the uncharacterized strains of PVX was used as a template for the synthesis of first-strand cDNA primed with an oligo(dT) linker-primer, dT/Kpn, as described previously (Santa Cruz & Baulcombe, 1993). Primers used for cDNA synthesis, PCR amplification and sequencing were prepared on an Applied Biosystems DNA synthesizer model 391. First-strand cDNA products were used as templates for 20 cycle PCR amplifications using a PVX-specific primer OX6 and L/Kpn, which allows amplification of cDNAs primed using dT/Kpn. The primer OX6 was designed to hybridize to the sequence immediately upstream of the coat protein gene that, on the negative strand viral RNA, is believed to function as a promoter for subgenomic RNA synthesis (Skryabin et al., 1988). All of the strains of PVX included in this analysis yielded a single cDNA product when amplified with the primers OX6 and L/Kpn. Amplified cDNAs were ligated to EcoRV-digested pKR (Waye et al., 1985) as described by Santa Cruz & Baulcombe (1993). Manipulations of cDNAs and plasmids were performed using standard techniques (Sambrook et al., 1989).

Plasmid clones carrying PVX coat protein cDNAs were used as templates for nucleotide sequence determination using Sequenase 2.0 (USB) according to the manufacturer’s instructions. The design of primers for sequencing across the PVX coat protein genes was based on published sequence data (Huisman et al., 1988; Kavanagh et al., 1992; Goulden et al., 1993). The sequence was determined across both strands of the viral cDNAs. For the PVX strains DY, EX, NL4, WS2, XA and XS two cDNA clones, derived from independent RNA preparations, were sequenced. For the strains KP and NL1 only one cDNA clone was sequenced. Analysis and manipulation of nucleotide and amino acid sequence data were performed using the UWGCG program (Devereux et al., 1984).

Nucleic acid sequence similarity between the PVX coat protein genes ranged from 77–99%. No differences in predicted amino acid sequence were found between independent coat protein cDNAs that were used in this study along with four previously reported PVX coat protein sequences. Subsequent analyses of amino acid sequences were based on the numbering of the 237 amino acid protein as indicated in the alignment (Fig. 1). Overall the primary structure of the coat protein was highly conserved between strains with a minimum of 90% of identical residues for any pairwise comparison. The coat proteins of strains DX and KP were identical to one another, and to a previously described Dutch strain PVX-X3 (Huisman et al., 1988).

The relationships between coat proteins of the different strains are shown in Fig. 2. The overall differences in coat protein primary structure were sufficient to permit a classification into two major types, X and B. The type X coat proteins were very closely related to one another, showing 97–100% identity at the amino acid level, and were all 237 amino acids in length. The type B proteins were less closely related to one another and could be further divided into the Bi and Bii subtypes on the basis of protein length (Bi, 248 amino acids: DY, EX, NL4; Bii, 236 amino acids: CP4, HB, WS2) and overall

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nil</th>
<th>XS</th>
<th>DX</th>
<th>KP</th>
<th>UK3</th>
<th>XA</th>
<th>DY</th>
<th>EX</th>
<th>WS2</th>
<th>CP4</th>
<th>Nil</th>
<th>HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>(nx)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>(Nb)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

* Strain susceptibility; R, resistant.
† Strain of PVX used for inoculation. The strains were originally isolated in the following countries: DX, DY, EX, KP and UK3, UK; Nil and Nil, The Netherlands; CP4, Peru; HB, Bolivia; WS2, USA; XA and XS, unknown.
‡ Genotype of potato cultivar.
§ The genotype of the Rx cultivar Cara with respect to \(Nx\) and \(Nb\) is unknown.
¶ Strain group according to the classification of Cockerham (1955).
Fig. 1. Alignment of 12 PVX coat protein sequences generated using the program PILEUP. Lines one to six represent strains avirulent on \(N_x\) gene-carrying potatoes; lines seven to twelve represent \(N_x\) resistance-breaking strains. Residues where all strains avirulent on \(N_x\) cultivars differ from the \(N_x\) resistance-breaking strains are indicated in bold; non-conservative substitutions are marked with an asterisk. The numbering of amino acid residues is based on the 237 amino acid long proteins (upper six lines).
similarity. This distinction also correlated with geographical origin since strains with subtype Bi coat proteins all derived from Europe whereas strains with subtype Bii coat proteins were from either North or South America.

The distinction between type B and type X coat proteins correlated with the ability or inability, respectively, of the different strains to overcome \( Nx \)-mediated resistance. Thus the group one and three strains, which induce the \( Nx \)-mediated HR, had type X coat proteins whereas strains belonging to groups two and four, which overcome \( Nx \)-mediated resistance, had type B coat proteins. In contrast no correlation was observed between coat protein primary structure and the ability of strains to overcome \( Nb \)-mediated resistance suggesting that the viral coat protein is unlikely to determine the \( Nb \)-mediated resistance response. The ability to overcome \( Rxl \) resistance shown by PVX strain HB is conferred by a lysine residue at position 122 of the strain HB coat protein; strains of PVX avirulent in \( Rxl \) potatoes have a threonine residue at this position (Goulden et al., 1993). The novel strains of PVX described here all failed to overcome \( Rxl \)-mediated resistance and all had a threonine residue at position 122 of their coat proteins.

In all there were 14 amino acid positions where all of the type X proteins differed from all of the type B proteins (Fig. 1). A previous analysis of \( Nx \) resistance-breaking mutants of the strain DX showed the coat protein region around residues 62–78 to be particularly important in determining the outcome of interactions between PVX and \( Nx \) gene-bearing potatoes (Kavanagh et al., 1992; Santa Cruz & Baulcombe 1993). The current analysis identified four of the 14 polymorphisms associated with the delineation between the type X and B coat proteins within this region. Analysis of mutant and recombinant forms of the PVX coat protein revealed that at least five of the 14 variable residues have no role in the interaction leading to \( Nx \)-mediated resistance. The three variable residues within the amino-terminal 20 amino acids of the coat protein are not essential for induction of \( Nx \)-mediated resistance; they are absent in the, UK3 derived, deletion mutant TXD9 that is avirulent in \( Nx \) gene-carrying potatoes (Chapman et al., 1992; Santa Cruz, 1993). We can also discount there being a role for the residues at positions 166 and 227 based on the analysis of a hybrid form of PVX (KC4) that is avirulent on \( Nx \) gene-carrying potatoes despite its having the carboxy-terminal domain of the coat protein (amino acid positions 137–237) of the virulent strain CP4 (Santa Cruz, 1993).

Secondary structure predictions based on the method of Chou & Fasman (1974), using the program ‘PEPTIDESTRUCTURE’, showed differences in the predicted secondary structures between type X and type B coat proteins in the amino-terminal variable-length domain and in the region of the amino acid substitution at position 227. However, both of these regions of the coat protein are outside the region implicated in \( Nx \)-mediated interactions.

Based on the classification of Kamer & Argos (1984), only four of the 14 amino acid differences that distinguish type X coat proteins from type B proteins were non-conservative substitutions. Of these non-conservative substitutions one was at position 20 and thus was outside the region implicated in induction of the \( Nx \)-mediated response. Two of the non-conservative substitutions, occurring at positions 64 and 128, resulted in the replacement of an uncharged residue as found in the type X proteins with charged residues in the type B proteins. The non-conservative substitution, occurring at position 227, although predicted to alter local secondary structure, was also outside the region believed to determine the outcome of interactions between PVX and \( Nx \) hosts.

The role of local charge variation as a possible determinant of the outcome of interactions between viral proteins and plants carrying resistance genes has been noted in other plant–virus pathosystems (Meshi et al., 1988, 1989; Calder & Palukaitis, 1992). However, a previous analysis of \( Nx \)-resistance breaking derivatives of the strain DX showed that there was no correlation between mutations affecting interactions with potatoes.

---

**Fig. 2.** Relationships between PVX coat proteins. Horizontal distances indicate degree of relatedness. The dendrogram was generated using the program PILEUP with a threshold value of one. Coat protein (sub)types are indicated to the right of the figure.
carrying the Nx gene and altered charge of the coat protein (Santa Cruz & Baulcombe, 1993). For the interaction between tobacco mosaic virus and the N' gene of N. sylvestris the viral coat protein is known to be responsible for eliciting the host response (Knorr & Dawson, 1988; Culver & Dawson, 1989; Culver et al., 1991). A systematic study of coat protein amino acid substitutions has demonstrated that N'-mediated recognition requires the maintenance of the coat protein tertiary structure but with weakened subunit-subunit interactions (Culver et al., 1994).

The analysis of natural variation occurring between the coat proteins of strains of PVX indicated a clear correlation between protein primary structure and the outcome of interactions between PVX and potatoes carrying the Nx gene. We are currently investigating the role of specific amino acid residues in the coat protein of PVX strain UK3 in order to determine the precise features required for induction of the Nx-mediated response.

The Sainsbury Laboratory is supported by the Gatsby Charitable Foundation. Work with PVX was carried out under licence from MAFF (PHF 1420/37/33). We are grateful to Gill Brewer, Chris Cupers, Esteban Hopp and Phil Jones for providing the PVX strains used in this analysis. We also thank Gill Fraser and Fiona Carr for assistance with graft inoculations and Sean Chapman for critical reading of the manuscript.

References


(Received 24 January 1995; Accepted 20 April 1995)