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Functionally Homologous Host Components Recognize Potato Virus X in *Gomphrena globosa* and Potato

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All known isolates of potato virus X (PVX), with the exception of a South American isolate PVX_{HB}, induce an extreme resistance response on potato carrying the *Rx* gene and elicit the production of necrotic lesions on *Gomphrena globosa*: PVX_{HB} establishes systemic infection on *Rx* genotypes of potato and infects the inoculated leaf of *G. globosa* without lesion formation. Previously, we have shown that the *Rx*-mediated resistance is affected by a feature of the coat protein that depends on the presence of a threonine residue at position 121 in the coat protein of PVX_{CP4} and that the resistance is an induced response expressed in protoplasts of potato with the *Rx* genotype. In this study, we provide evidence, based on the analysis of PVX_{CP4}/PVX_{HB} hybrids, that the elicitation of lesions on *G. globosa* also requires the presence of a threonine residue at position 121 of the viral coat protein. The lesion-forming phenotype was not associated with the ability of the viral isolate to accumulate in the infected plant. We therefore propose that there is a homologous component of both potato carrying *Rx* and *G. globosa* that interacts with a feature of the PVX coat protein and, following the interaction, activates an induced response in the plant cell.

INTRODUCTION

Induced disease resistance in plants is initiated by a highly specific interaction that is defined genetically as an interaction between the avirulence (*avr*) gene of the pathogen and the resistance (*R*) gene of the host plant (Keen, 1990). In contrast to the highly specific nature of this initiation process, the induced processes in the plant are often nonspecific so that, for example, a viral interaction may induce production of antifungal enzymes (Bowles, 1990) and the elicitation of systemic acquired resistance to diverse pathogens in the inoculated plant (Kuć, 1982; Ward et al., 1991). In many instances, these induced responses are associated with elicitation of a hypersensitive response (HR) at the site of inoculation, although there are examples in which the induced response is not associated with the necrosis (Jakobek and Lindgren, 1993). Ultimately, understanding the control and expression of these generalized disease defense mechanisms in the plant will require a characterization of different *avr–R* gene interactions. Resistance genes controlling induced resistance have eluded molecular analysis until now. However, over the last few years there has been considerable progress in the analysis of the *avr* genes of various plant pathogens, including bacteria, fungi, and viruses (Keen, 1990; De Wit, 1992).

One surprising finding, made possible by the molecular cloning of *avr* genes from bacteria, has been that Avr functions elicit defense responses even in plant species which are nonhosts for the pathogen from which the *avr* gene was cloned (Whalen et al., 1991; Dangl et al., 1992). This finding has led to the suggestion that some *R* genes, which are normally detected only when plant cultivars have pathovar-specific resistance, may have functional homologs in nonhost plants and may even contribute to the nonhost status of the plant for that pathogen. It had been thought previously that the pathovar-specific and nonhost resistances would involve different types of defense mechanism (Heath, 1991a, 1991b).

In some instances, elicitation of defense reactions to bacterial and fungal pathogens is carried out directly by the product of the *avr* gene. Fungal elicitor molecules that are the products of the *avr* genes are produced in races of *Fulvia fulvum* (De Wit, 1992; Van den Ackerveken et al., 1992), which are avirulent on tomato plants carrying the *Cf9* gene. In bacterial systems, the AvrBs3 protein is thought to interact with the product of *Bs3* in pepper and, as a result, to elicit resistance to *Xanthomonas campestris* pv *vesicatoria* (Herbers et al., 1992). There is also at least one instance in which the elicitor is not the direct product of the *avr* gene. This example involves *avrD* in *Pseudomonas syringae* pv *tomato* in which the elicitor of resistance on soybean carrying the *Rpg4* locus (Kobayashi et al., 1989; Keen et al., 1990) comprises two γ lactones, which are thought to be produced enzymatically by the product of *avrD* (Keen et al., 1993).

In viral systems the identification of Avr determinants is less extensive than with fungal and bacterial systems because, at least to some extent, it is more difficult to carry out a genetical analysis of viral determinants involved in resistance interactions. The starting point for the analysis of resistance

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interactions in viral systems has been the identification of viral factors associated with resistance breaking. In tobacco mosaic virus (TMV), each of the viral genes has been associated with the breaking of resistance conferred by different genes in the host: the gene for the putative replicase component affects resistance breaking of *Tm1* on tomato (Meshi et al., 1988) and, through a separate domain, the resistance mediated by the *N* gene on tobacco (Padgett and Beachy, 1993); the gene for the movement protein affects the resistance-breaking property of an isolate on *Tm2* on tomato (Meshi et al., 1989); and the coat protein affects resistance breaking of the *N'* gene on tobacco (Saito et al., 1987; Knorr and Dawson, 1988). In potato virus X (PVX), the coat protein gene affects the ability of an isolate to overcome *Nx* and *Rx* in potato (Kavanagh et al., 1992). However, the association of a viral gene or its product with resistance breaking does not necessarily equate with an avirulence determinant: resistance breaking could be due to production of an avirulence determinant or of a virulence factor that causes suppression of avirulence.

There are two virus resistance genes that control induced resistance mechanisms and for which the viral Avr functions have been identified. It is thought that these viral Avr determinants are like Avr9 and AvrBs3 in that the product of the *avr* gene is also the elicitor of resistance. The first viral Avr determinant to be characterized was the coat protein of some isolates of TMV. This protein is the avirulence function for the *N'* gene of tobacco (Culver and Dawson, 1989, 1991; Pfitzner and Pfitzner, 1992): *N'* cultivars of tobacco inoculated with avirulent isolates of TMV produce a typical HR involving the formation of local necrotic lesions at the sites of virus inoculation. The other example involves the coat protein of PVX, which is an avirulence determinant on potato carrying the *Rx* gene (Kavanagh et al., 1992; Köhm et al., 1993). The resistance conferred by *Rx* leads to suppression of virus multiplication in the inoculated cell (Adams et al., 1986; Saladrigas et al., 1990; Köhm et al., 1993) but under most conditions there is no obvious HR. This type of resistance has been referred to as extreme resistance (Tozzini et al., 1991). The conclusion that *Rx* mediates an induced response is based on the observation that potato protoplasts carrying *Rx* and infected with an avirulent strain of PVX become modified such that they do not support efficient accumulation of cucumber mosaic virus or an isolate of PVX otherwise able to overcome the *Rx*-mediated resistance (Köhm et al., 1993). We have recently carried out an extensive analysis of the PVX coat protein gene and have identified a threonine residue at position 121 of the coat protein that is necessary for the avirulence function on potato carrying *Rx* (Goulden et al., 1993).

In the work described in this paper, we have exploited unusual features of PVX_{HB}, which is a South American isolate of PVX. All isolates of PVX, except PVX_{HB}, are avirulent on potato with *Rx* and also induce necrotic lesions on *Gomphrena globosa*; PVX_{HB} is virulent on all cultivars of potato and infects the inoculated leaf of *G. globosa* without elicitating symptoms (Moreira et al., 1980). In this paper, we describe experiments designed to determine whether the elicitation of lesions on *G. globosa* is affected by the features of the PVX coat protein involved in the *Rx*-mediated interaction on potato.

RESULTS

A Necrosis Determinant on *G. globosa* in the Coat Protein Gene of PVX

To identify the PVX determinants that affect necrosis on *G. globosa*, we have tested a series of hybrid viral genomes incorporating parts of PVX_{HB} in other strains of PVX. PVX_{HB} is unique in that it infects *G. globosa* but fails to induce necrotic lesions (Moreira et al., 1980): all other known strains of PVX elicit necrotic lesion formation on this plant. The hybrids were constructed by modification of full-length cDNA clones described previously (Kavanagh et al., 1992; Goulden et al., 1993). The first hybrid genome to be tested was PVX_{KH1} in which the 3' part of the PVX_{UK3} genome, beyond nucleotide position 5250, was replaced with the homologous region from PVX_{HB} (Kavanagh et al., 1992). The symptoms induced by this hybrid PVX resembled those of PVX_{HB} in that it accumulated in the inoculated leaves of *G. globosa* without eliciting local lesions (data not shown). We have therefore concluded that all or part of the determinant of HR in *G. globosa* is in the region between nucleotide 5250 and the 3' end of the PVX_{UK3} genome which, as is shown in Figure 1, includes part of open reading frame (ORF) 3, ORF 4, and ORF 5 (coat protein gene) of the PVX genome.

The analysis was extended by testing a series of hybrids incorporating parts of PVX_{HB} and PVX_{CP4}. PVX_{CP4}, like PVX_{UK3}, elicits local lesions on *G. globosa* but, being more similar to PVX_{HB} in nucleotide sequence (Goulden et al., 1993), could be incorporated more easily than PVX_{UK3} into hybrid viral genomes based on PVX_{HB}. The PVX_{CP4} component of these hybrids was derived from a transcription clone that includes the full-length cDNA of PVX_{CP4} (Goulden et al., 1993). The in vitro transcripts of this cDNA are infectious on various plant species and elicit the types of symptoms described previously for PVX_{CP4} (Jones, 1985): on *Nicotiana clevelandii*, there was a strong well-defined mosaic with necrotic flecks, whereas on *G. globosa* necrotic local lesions developed on the inoculated leaf.

The PVX_{CP4}/PVX_{HB} constructs described in Figure 1B were designed to test whether there is a local lesion determinant in the coat protein gene (ORF 5) of PVX_{CP4}. The construct PVX_{HC1} was a substitution into PVX_{HB} of the part of the PVX_{CP4} coat protein gene containing all seven coding sequence differences in the coat protein genes of these two strains (Goulden et al., 1993). The other constructs were substitutions of smaller parts of the PVX_{CP4} coat protein gene into PVX_{HB} and were to test the role of particular coding sequence differences in the elicitation of a response on *G. globosa*.

The experiments were carried out by inoculation of transcripts from the hybrid cDNA clones to *N. clevelandii* and

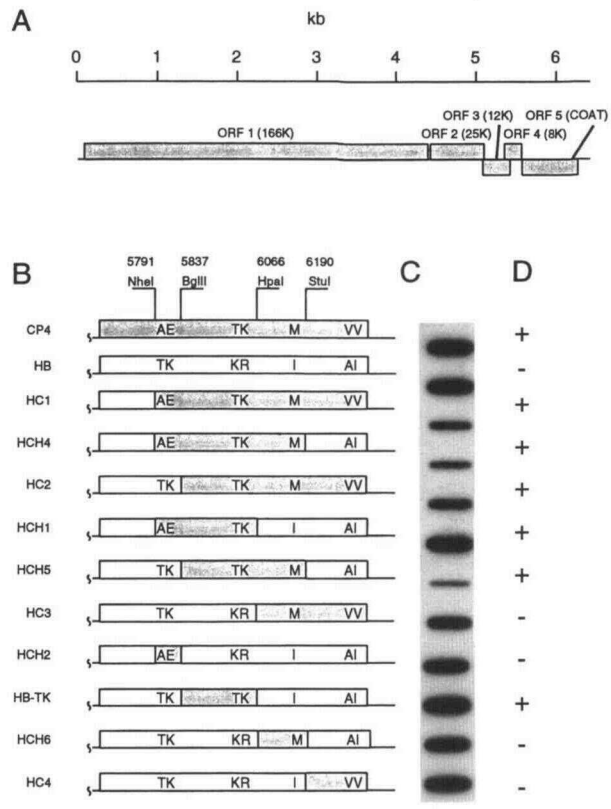

Figure 1. PVX_{HB} Hybrid Genomes Inoculated to *G. globosa*.

PVX constructs based on the genome of PVX_{HB} were inoculated to *G. globosa* and inspected for lesion formation at 15 days postinoculation. RNA samples were taken for slot blot analysis of PVX RNA accumulation.

(A) The genome organization of PVX indicated to scale and showing the relative size of the five ORFs. The relative position of features on the viral genome can be inferred by reference to the scale in kilobases.

(B) The composition of the hybrid genomes in the region of the coat protein gene shown with the PVX_{HB} components indicated by the open boxes and PVX_{CP4} as shaded areas. The diagram also shows the sequence coordinates and identity of restriction enzyme sites used in the construction of the cDNA hybrids and, using the single-letter code, the coat protein residues that are polymorphic in PVX_{CP4} and PVX_{HB}.

(C) Slot blot analysis of RNA from leaves of *G. globosa* infected with the PVX genomes shown in **(A)**. The RNA samples were isolated from the inoculated leaves at 14 days postinoculation, filtered onto Hybond N membranes, and hybridized with a ^{32}P-labeled PVX-specific probe, as indicated in Methods.

(D) The lesion forming phenotype of the viral constructs. The symbols indicate whether (+) or not (–) necrotic lesions were induced on the leaves of *G. globosa*.

subsequent passage of the progeny virus to *G. globosa*. The effects induced by four of the hybrid isolates of PVX (PVX_{HC3}, PVX_{HCH2}, PVX_{HCH6}, and PVX_{HC4}; Figure 1B) were similar to those of PVX_{HB}, as shown in Figure 2A: these isolates failed to elicit lesions on *G. globosa*, even when repeatedly inoculated and regardless of whether the plants were maintained at 15 to 20°C or 25 to 30°C. These hybrids all had the PVX_{HB} identity at residues 121 and 127 of the coat protein (K and R, respectively). The rest of the hybrids (PVX_{HC1}, PVX_{HCH4}, PVX_{HC2}, PVX_{HCH1}, PVX_{HCH5}, and $PVX_{HB\text{-}TK}$; Figure 1), with the PVX_{CP4}-derived T and K residues at positions 121 and 127, all elicited necrotic local lesions. However, the lesions differed from those on *G. globosa* inoculated with PVX_{CP4}. The PVX_{CP4}-induced lesions appeared 3 to 4 days postinoculation on plants maintained at 15 to 20°C and were 1 to 2 mm in diameter. By 8 to 10 days postinoculation, the lesions formed a necrotic center and a large red border with a diameter of typically 4 to 5 mm (Figure 2B) extending to 8 mm in some instances. The lesions continued to expand and adjacent lesions eventually coalesced to form broader areas of necrotic tissue (Figure 2C). With younger plants inoculated to produce more than 50 local lesions per leaf, this coalescence resulted in confluent necrosis of the leaf by 15 days postinoculation. At 25 to 30°C, the initial appearance of the lesions was the same as at 15 to 20°C, but they failed to expand, even by 1 month postinoculation. The lesions induced by the six hybrids referred to above developed more slowly and spread less readily than the PVX_{CP4} lesions. The lesions elicited by the hybrid strains appeared at 8 to 10 days postinoculation on plants maintained at 15 to 20°C (shown in Figure 2D for $PVX_{HB\text{-}TK}$), and the necrotic region had a well-developed red border. The necrotic region was smaller than in the lesions with PVX_{CP4}, was never more than 1 to 2 mm in diameter, and did not coalesce, even when the lesions were close together on the leaf. At 25 to 30°C, the necrotic centers of the lesions were smaller (1 mm diameter maximum) than at 15 to 20°C, and, when induced by hybrids PVX_{HC1}, PVX_{HCH4}, and PVX_{HCH1}, the necrotic regions were eventually repaired so that at 1 month postinoculation the lesions appeared chlorotic rather than necrotic.

To determine whether the four hybrids that did not induce necrotic lesions were able to accumulate on *G. globosa*, RNA was extracted from the inoculated leaves and subjected to slot blot analysis. The results showed that the nonnecrogenic isolates, including PVX_{HB}, each accumulated at similar levels (Figure 1C). Comparison with RNA dilution standards confirmed that there was no more than twofold variation in the accumulation level of those RNAs. PVX RNA was also recovered from *G. globosa* leaves with virus-induced lesions, but only in the regions around the lesions (Figure 1C): regions between lesions were PVX free, as assessed by RNA gel blot analysis and ELISA (M.G. Goulden, unpublished data).

To confirm the identity of viral RNAs in the leaves of *G. globosa*, polymerase chain reaction (PCR)–amplified cDNAs encompassing the coat protein region between nucleotides 5617 and 6405 from each construct were subjected to restriction enzyme analysis. This test showed that viral cDNAs derived from the host had restriction sites identical to those in PCR products amplified from the corresponding transcription clone. The restriction sites used in this analysis were MscI (5804), PvuII (5987), and NaeI (6078) that were diagnostic of PVX_{CP4}-

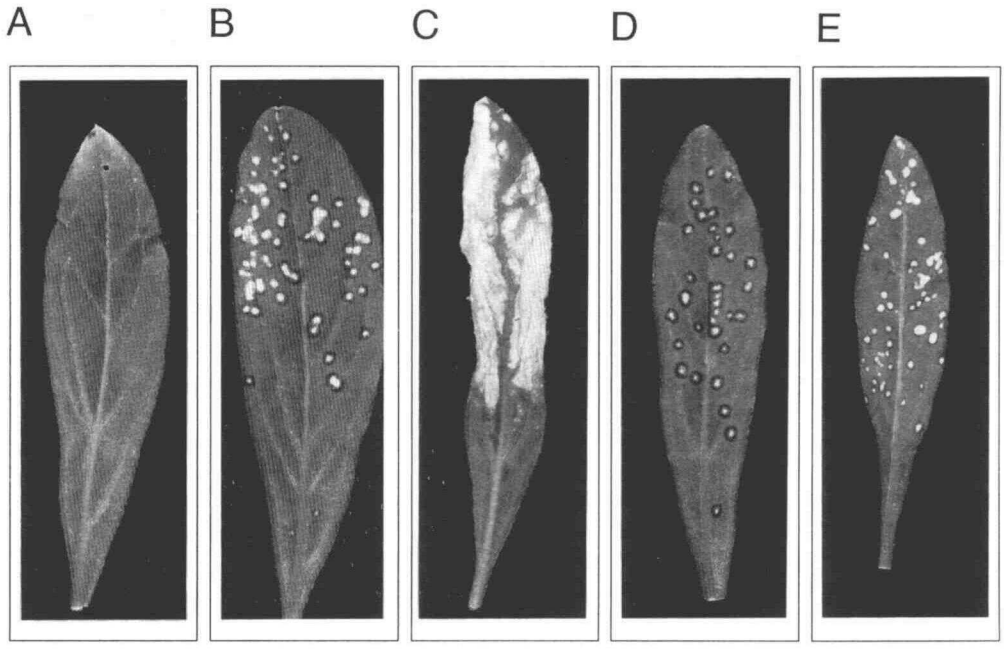

Figure 2. Leaves of *G. globosa* Inoculated with Hybrid Forms of PVX.

Leaves of *G. globosa* were inoculated with extracts of *N. clevelandii* infected with the indicated isolate or hybrid form of PVX. These examples illustrate the range of responses to PVX on the leaves of *G. globosa.*

(A) Inoculated with PVX_{HB} and photographed 14 days postinoculation.

(B) Inoculated with PVX_{CP4} and photographed 14 days postinoculation.

(C) Inoculated with PVX_{CP4} and photographed 23 days postinoculation.

(D) Inoculated with $PVX_{HB\text{-}TK}$ and photographed 14 days postinoculation.

(E) Inoculated with $PVX_{CP4\text{-}KR}$ and photographed 14 days postinoculation.

derived sequence and PstI (5711 and 6158 in PVX_{CP4} and 5711, 6024, 6158, and 6329 in PVX_{HB}) (Goulden et al., 1993).

The Identity of Coat Protein Codon 121 Affects Elicitation of Local Lesions on *G. globosa*

As indicated above, the hybrid PVX genomes able to elicit local lesions on *G. globosa* were those with T and K at codons 121 and 127 in the coat protein gene. These constructs also had 28 other noncoding changes from the PVX_{HB} genome located, in the cDNA, between the BglII and HpaI sites indicated in Figure 1 (Goulden et al., 1993). To confirm that the coding sequence differences from the PVX_{HB} genome were responsible for the altered phenotype on *G. globosa*, two additional mutant versions of PVX_{HB} were tested by inoculation to *G. globosa.* $PVX_{HB\text{-}TR}$ (Goulden et al., 1993) had a single nucleotide change relative to PVX_{HB}, causing codon 121 of the coat protein gene to specify a T rather than a K residue, as occurs in PVX_{HB}, as shown in Figure 3A. $PVX_{HB\text{-}KK}$ was derived from $PVX_{HB\text{-}TK}$, by once again changing codon 121, but to specify a K rather than a T residue (Figure 3A).

The transcripts of these two mutant constructs were infectious on *N. clevelandii*, although, as described previously (Goulden et al., 1993), they were unable to spread from the inoculated leaf and had a specific infectivity three to four orders of magnitude less than their progenitor isolates PVX_{HB} or $PVX_{HB\text{-}TK}$. However, despite this reduced infectivity, the two mutant constructs were able to accumulate in the inoculated leaves of *G. globosa* (Figure 3B). $PVX_{HB\text{-}KK}$ failed to induce lesions on the leaves of *G. globosa.* This result therefore implicated the threonine codon at position 121 of the coat protein gene in the lesion-inducing phenotype of PVX_{CP4} because that codon was the only difference between $PVX_{HB\text{-}KK}$ and its lesion-inducing progenitor $PVX_{HB\text{-}TK}$. The second mutant, $PVX_{HB\text{-}TR}$, induced lesions on *G. globosa*, whereas PVX_{HB}, with only a single nucleotide difference at codon 121, failed to elicit lesions. These results reinforce the conclusion that the coat protein residue at codon 121 is implicated in the lesion-inducing phenotype of PVX_{CP4}. The result also indicates that lesion formation on *G. globosa* was not affected by the noncoding sequence differences in the coat protein genes of PVX_{CP4} and PVX_{HB}.

The mutants $PVX_{CP4\text{-}KK}$, $PVX_{CP4\text{-}KR}$, and $PVX_{CP4\text{-}TR}$ are the reciprocal forms of the PVX_{HB} constructs described above (Figure 3A) with the central part of the PVX_{HB} coat protein substituted either in a wild-type or mutant form into the PVX_{CP4} genome. The $PVX_{CP4\text{-}KK}$ and $PVX_{CP4\text{-}TR}$ constructs,

like their PVX_{HB} homologs, had a lower specific infectivity than the wild-type viruses. However, the progeny virus of all three constructs accumulated (Figure 3B) and induced lesions on *G. globosa*. The lesions induced by $PVX_{CP4\text{-}TR}$ and $PVX_{CP4\text{-}KK}$ appeared 8 to 10 days postinoculation and were similar to those induced by the PVX_{HB}-based hybrids (Figure 2D). Those induced by $PVX_{CP4\text{-}KR}$ also appeared 8 to 10 days postinoculation and were similar in size to those induced by PVX_{CP4}, but they had a more necrotic center and a narrower red border at the edge of the lesion (Figure 2E). The sequence of the coat protein gene, in PCR-amplified cDNAs of the progeny virus, between nucleotides 5716 and 6405 was the same as in the original cDNA constructs.

The induction of lesions by $PVX_{CP4\text{-}TR}$ was consistent with the data from hybrids and mutants in the background of PVX_{HB}, suggesting a role of the threonine residue at position 121. However, to explain lesion induction by $PVX_{CP4\text{-}KK}$ and $PVX_{CP4\text{-}KR}$, we propose that PVX_{CP4} has an additional determinant for local lesion induction, which is independent of the determinant involving the T residue at position 121.

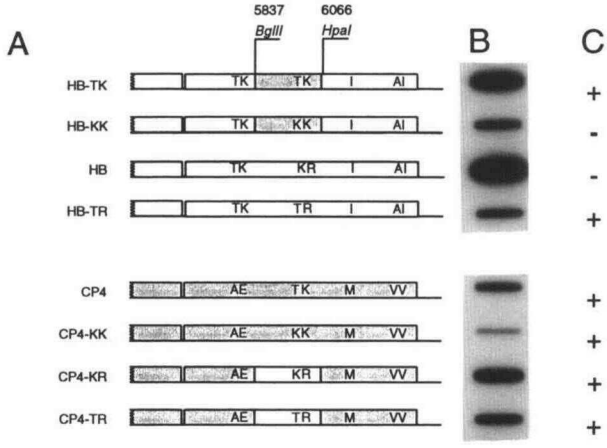

Figure 3. PVX_{HB} and PVX_{CP4} Hybrid and Mutant Genomes Inoculated to *G. globosa*.

PVX constructs based on the genome of PVX_{HB} or PVX_{CP4} were inoculated to *G. globosa* and inspected for lesion formation at 15 days postinoculation. RNA samples were taken for slot blot analysis of PVX RNA accumulation at 15 days postinoculation.

(A) The composition of the hybrid and mutant genomes in the region of the coat protein gene shown with the PVX_{HB} components is indicated by open boxes and PVX_{CP4} as shaded areas. The diagram also shows the restriction enzyme sites used in the construction of the cDNA hybrids and, using the single-letter code, the coat protein residues that are polymorphic in PVX_{CP4} and PVX_{HB}.

(B) Slot blot analysis of RNA from leaves of *G. globosa* infected with the PVX genomes shown in **(A)**. The RNA samples were isolated from the inoculated leaves at 15 days postinoculation, filtered onto Hybond N membranes, and hybridized with a ^{32}P-labeled PVX-specific probe, as indicated in Methods.

(C) The lesion-forming phenotype of the viral constructs. The symbols indicate whether (+) or not (−) necrotic lesions were induced on the leaves of *G. globosa*.

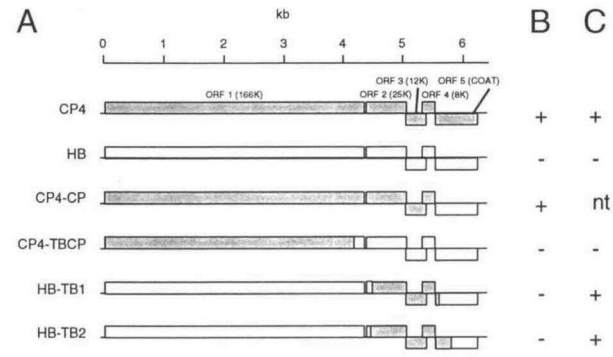

Figure 4. PVX_{HB} and PVX_{CP4} Hybrid Genomes Inoculated to *G. globosa*.

PVX constructs based on the genome of PVX_{HB} or PVX_{CP4} were inoculated to *G. globosa* and inspected for lesion formation at 15 days postinoculation.

(A) The composition of the hybrid and mutant genomes shown with the PVX_{HB} components is indicated by open boxes and PVX_{CP4} as shaded areas. The diagram also indicates the likely function or M_r of the virus-encoded gene products from ORFs 1 to 5. The relative position of features on the viral genome can be inferred by reference to the scale in kilobases.

(B) The lesion-forming phenotype of the viral constructs. The symbols indicate whether (+) or not (−) necrotic lesions were induced on the leaves of *G. globosa*.

(C) The lesion-forming phenotype of the viral constructs. The symbols indicate whether (+) or not (−) necrotic lesions were induced on the leaves of *C. amaranticolor*, and nt indicates not tested.

Localization of a Second Determinant of Local Lesion Formation in the Genome of PVX_{CP4}

The local lesion determinants of the PVX_{CP4} genome were further investigated by analysis of the effects of four PVX constructs on *G. globosa*, as shown in Figure 4. The substitution of the coat protein from PVX_{HB} into the genome of PVX_{CP4} created $PVX_{CP4\text{-}CP}$. In $PVX_{CP4\text{-}TBCP}$, the substitution of PVX_{HB} sequences into the genome of PVX_{CP4} extends downstream from position 4243 to include the C-terminal 70 amino acids of ORF 1, the triple block ORFs 2 to 4, and the coat protein gene. In $PVX_{HB\text{-}TB1}$, substitution of PVX_{CP4} sequences into the genome of PVX_{HB} extends between positions 4526 and 5684 and replaces the first 12 codons of the coat protein gene and the triple block ORFs 2 to 4, except the fifteen 5′ codons of ORF 2. In $PVX_{HB\text{-}TB2}$, the PVX_{CP4} sequences extend from 4500 to 5838, which replaces the triple gene block ORFs, as in $PVX_{HB\text{-}TB1}$, and codons 1 to 63 of the coat protein ORF.

Transcripts of these constructs were all infectious on *N. clevelandii*. On *G. globosa*, $PVX_{CP4\text{-}CP}$ induced lesions indistinguishable from those of $PVX_{CP4\text{-}KR}$ (Figure 2E). This confirmed the existence of a local lesion determinant outside the coat protein gene of the PVX_{CP4} genome. $PVX_{CP4\text{-}TBCP}$ did not induce lesions, thereby indicating that the determinant is within the 3′ region of ORF 1 or the triple gene block ORFs

2 to 4. However, the determinant is not within the triple gene block sequences substituted in $PVX_{HB\text{-}TB1}$ and $PVX_{HB\text{-}TB2}$, because neither of these constructs induced local lesions. This suggests that a second local lesion determinant of PVX_{CP4} may lie within the sequence replaced in $PVX_{CP4\text{-}TBCP}$ but outside the coat protein gene or the region replaced in $PVX_{HB\text{-}TB1}$ or $PVX_{HB\text{-}TB2}$. This region encodes the C-terminal 70 amino acids of the replicase and includes the intergenic region and the first five codons of ORF 2.

Symptoms Induced by Hybrid PVX Genomes on *N. clevelandii* and *Chenopodium amaranticolor*

The PVX_{CP4} and PVX_{HB} isolates differ in their ability to elicit symptoms on various species of Nicotiana, including *N. clevelandii*, and on the ability to elicit necrotic local lesions on *C. amaranticolor* (Moreira et al., 1980; Jones, 1985). For example, on *N. clevelandii*, PVX_{CP4} induced a strong well-defined mosaic pattern with associated necrotic flecks, whereas PVX_{HB}, as previously reported, induced a mild, barely perceptible mosaic pattern.

We failed to identify a discrete region affecting symptom function in *N. clevelandii*: none of the hybrids induced symptoms identical to the wild-type isolates. On *C. amaranticolor*, PVX_{CP4} induced strongly chlorotic lesions with central necrotic flecks, as shown in Figure 5A, and PVX_{HB} failed to induce lesions, as shown in Figure 5B. However, these differences were determined by parts of the viral genome other than the *G. globosa* local lesion determinants.

On *C. amaranticolor* as on *G. globosa*, $PVX_{CP4\text{-}TBCP}$, like PVX_{HB}, failed to induce lesions. However, $PVX_{HB\text{-}TB1}$ and $PVX_{HB\text{-}TB2}$ both induced chlorotic lesions (Figure 5C) that lacked the necrosis typical of lesions induced by PVX_{CP4} (Figure 5A). Based on these data (summarized in Figure 4C), we propose that triple gene block ORFs affect lesion induction on *C. amaranticolor*. However, it is also clear that other, unidentified components of PVX_{CP4} are also involved in lesion induction on *C. amaranticolor*, and it remains possible that these determinants might include the factors affecting lesion formation on *G. globosa*.

DISCUSSION

The analysis of hybrid and recombinant isolates of PVX has shown clearly that PVX_{CP4} encodes two determinants of necrotic lesion formation on *G. globosa*. The upstream determinant, between positions 4243 and 4500 of the viral genome, has not been fully characterized. It is not known, for example, whether this feature is an RNA elicitor of necrosis on *G. globosa* or whether the effect is mediated by PVX-encoded proteins. The downstream determinant has been localized to codon 121 of the coat protein gene. It is thought to elicit necrosis through a property of the coat protein, rather than the viral RNA, because the only mutations in PVX_{CP4} and PVX_{HB} isolates affecting the induction of necrosis on *G. globosa* are coding sequence changes (Figures 1 and 3). Our analysis of the lesion-inducing phenotype of the hybrid forms of PVX does not rule out that there is some overlap of the determinants of necrosis for *C. amaranticolor* and *G. globosa* (Figure 4C). However it does seem likely that there are determinants which act on *C. amaranticolor* and not on *G. globosa*.

In principle, the different lesion-inducing phenotypes of the PVX isolates could be due to a qualitative feature of the coat

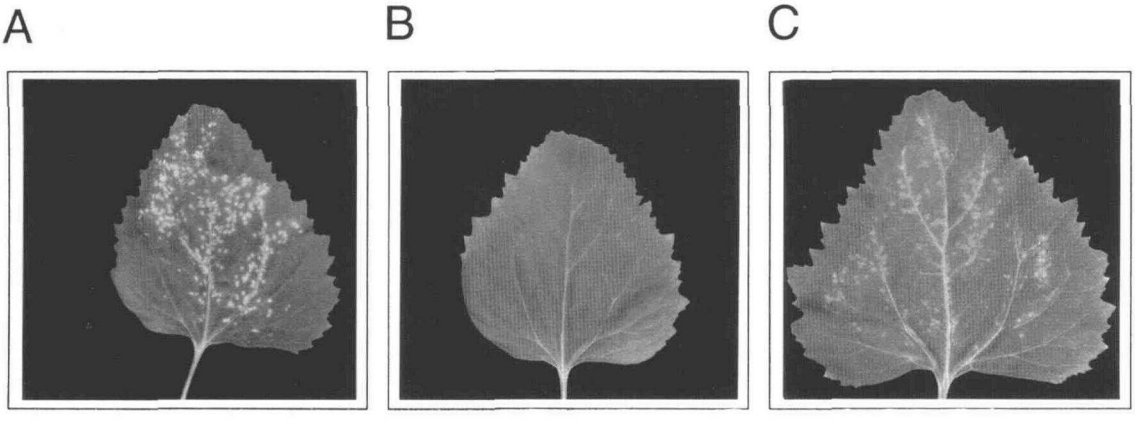

Figure 5. Symptom Effects of Mutant and Hybrid Forms of PVX Inoculated to Either *C. amaranticolor* or *N. clevelandii*.

Leaves of *C. amaranticolor* were inoculated with extracts of *N. clevelandii* injected with the indicated forms of PVX and photographed at 14 days postinoculation.

(A) PVX_{CP4}.

(B) PVX_{HB}.

(C) $PVX_{HB\text{-}TB1}$.

protein or to an effect of the mutation at position 121 of the coat protein on the ability of the virus to accumulate and spread in the infected plant. In work reported elsewhere (Goulden et al., 1993), we have shown that mutation of the PVX coat protein at codon 121 or 127 impairs the ability of the virus to accumulate and spread in the infected plant (i.e., the viral fitness). The debilitating effect of the mutation at position 121 could be compensated for by a second mutation at position 127 and vice versa. Thus, isolates were fully fit if there were T and K codons at these two positions as in PVX_{CP4} or K and R as in PVX_{HB}. The isolates with reduced fitness had either the T and R or K and K combinations of these two codons (Goulden et al., 1993). The specific infectivity on *G. globosa* of these mutant forms of PVX and the level of accumulation of PVX RNA (Figure 3B) indicate that the debilitating effects of the mutations also apply on *G. globosa*. However, these effects on viral fitness do not apparently interfere with the ability of PVX isolates to elicit necrosis on *G. globosa*: the necrogenic mutants $PVX_{HB\text{-}TR}$ and $PVX_{CP4\text{-}TR}$ both had the T and R combination of codons at positions 121 and 127 and consequently displayed the reduced fitness phenotype. We therefore propose that lesion formation on *G. globosa* is not affected by viral fitness. A more likely possibility is that the plant has a component which recognizes a feature of the PVX coat protein affected by sequence identity at position 121. This component presumably controls the biochemical changes in *G. globosa* leading to the necrotic response, dependent on the presence of the appropriate form of the PVX coat protein.

The PVX recognition functions in *G. globosa* and in *Rx* genotypes of potato are probably interacting with the same feature of the viral coat protein: both are affected by a threonine-to-lysine mutation at position 121. We cannot conclude that the threonine is involved directly in the interaction with the plant product because physical analyses of the viral coat protein have indicated that position 121 is not exposed on the surface of the molecule (Baratova et al., 1992a, 1992b). The most likely possibilities are that the threonine-to-lysine change has a secondary effect on the structure of the molecule or that the interaction involves a noncapsid form of the molecule.

The proposition that the same feature of the PVX coat protein is recognized in both *G. globosa* and potato has prompted us to ask whether this is a chance similarity or whether there is a functional homolog of *Rx* in these unrelated plants. If the *Rx*-mediated resistance is quite distinct mechanistically from resistance mechanisms associated with the elicitation of the HR, the chance effect would be the more likely explanation. Conversely there would be support for the idea of functional homologs in these plants if the response to PVX on *G. globosa* was biochemically similar to the response in potato carrying *Rx*.

There is no question that components of the *Rx*-mediated response and the response of *G. globosa* are distinct: *Rx*-mediated resistance blocks virus accumulation in the inoculated cell (Adams et al., 1986; Saladrigas et al., 1990; Tavantzis, 1990; Köhm et al., 1993), whereas on *G. globosa* there is evidently accumulation of virus in the inoculated cell and the surrounding area (Figures 1 and 3). However, the nature of

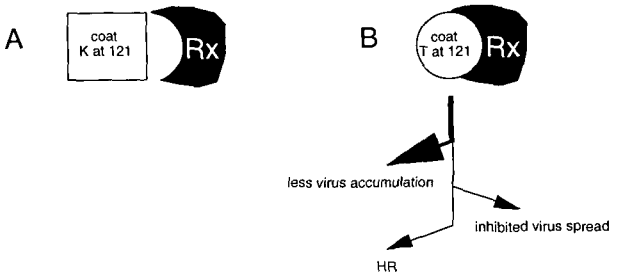

Figure 6. A Model of the Mechanism of *Rx*-Mediated Resistance.

The resistance is shown as a two-stage process with recognition being separate from the response.

(A) The compatible interaction. Recognition of the PVX fails because of the absence of threonine at position 121 of the coat protein. In the absence of recognition, there is no response.

(B) The incompatible interaction. The product of *Rx* recognizes the PVX coat protein with threonine at position 121. The thick line indicates a component of the response that prevails after manual inoculation of potato carrying *Rx*. The thin line indicates the responses active in graft inoculated potato if there are mutations in the coat protein affecting the coat protein structure (Goulden et al., 1993) in potato carrying $Rx_{acl}{}^{n}$ or in *G. globosa* if the PVX recognition component is a homolog of *Rx*.

this *Rx*-mediated effect may mask secondary effects that are more similar to the HR-like response observed on *G. globosa*.

Several lines of evidence indicate that this masking effect does take place and that, under defined experimental conditions, there can be secondary effects associated with the *Rx*-mediated resistance. For example, we have described a mutant form of PVX that can overcome *Rx*-mediated resistance at the protoplast level but that is still avirulent when inoculated to plants (Goulden et al., 1993). We interpret that result to indicate that *Rx*-mediated resistance is a multicomponent process of which the effect observed in protoplasts is just one. There is also genetical evidence from potato that *Rx*-mediated resistance may include components which do not affect accumulation of the virus in the inoculated cell: the allele of *Rx*, $Rx_{acl}{}^{n}$ is associated with the HR rather than the extreme resistance phenotype (Cockerham, 1970). Finally, there are reports that if potato carrying *Rx* is graft inoculated, there is low-level accumulation of PVX in the systemic tissue and some necrosis (Benson and Hooker, 1960; Bagnall, 1961). In graft-inoculated potato, a PVX-infected scion from a susceptible plant is spliced to the stock of a resistant plant and, following from formation of the graft union, there would be continuous vascular flow of inoculum to the resistant plant. It is therefore likely under graft inoculation conditions that the primary effect of *Rx*-mediated resistance would be overcome and that there would be elicitation of the secondary components of the *Rx*-mediated resistance mechanism.

The diagram in Figure 6 is our current model for the mechanism of *Rx*-mediated resistance based on the observations described above. We propose that the resistance is a two-phase process and that the branches of the response phase are

activated differentially depending on the nature of the components in the recognition reaction and on the physiological condition of the inoculated plant. The *G. globosa* response to PVX with a T residue at the position 121 of the coat protein could be accommodated in this model if the PVX recognition component in *G. globosa* is a functional homolog of *Rx* in which, like Rx_{acl}^{n}, it has little or no effect on the response component leading to suppression of virus accumulation (Figure 6).

There are several precedents from other pathosystems that reinforce the plausibility of the model and the notion that homologs of *Rx* may be present even in plants which are nonhosts for PVX. For example, in nonviral pathosystems it is thought that there is a multicomponent response mediated by interaction of *avr* in the pathogen and *R* in the host (Kiraly et al., 1972; Jakobek and Lindgren, 1993). In addition to the HR, there are several other components to the *avr*-induced response in a resistant plant. The other components include induction of antifungal enzymes, production of antibacterial metabolites, and the activation of other poorly understood processes that prevent growth of the pathogen (reviewed recently by Lamb et al., 1992).

If there is a homologous function of *Rx* in *G. globosa*, the situation would be similar to that described in bacterial pathosystems in which there are Avr recognition functions in plants outside the host range of the avirulent pathogen (Whalen et al., 1988, 1991; Dangl et al., 1992). These observations with bacterial pathosystems have prompted the reevaluation of the concept of host range limitation in plants to include the idea that a plant may be a nonhost for a pathogen because it recognizes the products of the pathogen as if they are avirulence determinants (Heath, 1991a, 1991b). It may be necessary to extend this reevaluation to include virus resistance if, as proposed above for *Rx*, there are homologs of genes controlling extreme resistance in unrelated plants, including "nonhosts" for the virus recognized by the resistance gene product. It is interesting to consider how, until the discovery of the resistance-breaking isolate of PVX (Moreira et al., 1980) and the genetic analyses of *Rx* (Cockerham, 1970), the species *Solanum andigena* and *S. acaule*, from which *Rx* is derived (by introgression), would have been considered as nonhosts of PVX: the inoculated plants show no symptoms of infection and there is no accumulation of the infection or spread of the virus. It will be necessary to clone and characterize the *Rx* gene to determine whether the response to PVX on *G. globosa* and other nonhost plants is due to the action of molecular homologs of *Rx*.

METHODS

Viral cDNA Clones

The viral cDNA constructs used in this paper have all been described previously (Kavanagh et al., 1992; Goulden et al., 1993), with the exception of pTCP4-TBCP and pTHS-TB1 and -TB2. These plasmids were used to produce RNA inocula of the potato virus X (PVX) isolates $PVX_{CP4\text{-}TBCP}$, $PVX_{HB\text{-}TB1}$, and $PVX_{HB\text{-}TB2}$ produced by modification of pTCP4 or pTHS, which are the full-length cDNAs of PVX_{CP4} or PVX_{HB}, respectively. In pTCP4-TBCP, there is a replacement of the sequence between position 4243 and the 3′ end of the viral genome with the homologous sequence of pTHS. The constructs pTHS-TB1 and pTHS-TB2 had the sequences of pTHS replaced with the homologous region of pTCP4 between positions 4526 and 5634 and 4500 and 5838, respectively. The replacements were performed using recombination between restriction fragments, essentially as described for polymerase chain reaction (PCR) products by Jones and Howard (1991). The nucleotide sequence over the junction of PVX_{CP4}- and PVX_{HB}-derived cDNAs and of all PCR-derived cDNAs was confirmed directly.

Plant Inoculations

The mutant and hybrid viral cDNAs were transcribed into infectious RNA and inoculated to *Nicotiana clevelandii*, as described previously (Goulden et al., 1993; Köhm et al., 1993). An extract was produced from the inoculated leaves of infected plants at 10 days postinoculation by homogenization in 50 mM borate buffer (pH 8; 1 g/mL). The extracts were inoculated without dilution to *Gomphrena globosa* maintained in glasshouses with artificial illumination, when necessary to give an 18-hr day. The glasshouse was maintained at 15 to 20°C or 25 to 30°C, as indicated in the text. Slot blot analysis was performed as described previously (Goulden et al., 1993) using as a probe transcripts of pHB-RP, which is a cDNA clone of the 2.5 kb from the 3′ terminus of PVX_{HB}. The transcripts were of the nonviral strand and were used to detect the viral strand. Each slot was loaded with 1 μg of total RNA isolated by phenol/chloroform extraction of infected leaves, as indicated in the text. Quantification of the hybridization signal was by reference to a dilution series of RNA from infected leaves that was blotted and hybridized in parallel with the experimental samples.

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