Analysis of the Resistance-Breaking Determinants of Potato Virus X (PVX) Strain HB on Different Potato Genotypes Expressing Extreme Resistance to PVX

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ABSTRACT

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Different potato genotypes expressing extreme resistance to potato virus X were inoculated with potato virus X strains cp, CP4, and HB and with three PVX mutants constructed in vitro. Using these viral strains and mutants, the involvement of the coat protein, specifically the single amino acid residue at position 121 within this protein, in the resistance-breaking capacity of strain HB was confirmed for all resistance genes tested. We concluded that the extreme resistance expressed in *Solanum* × *chaucha*, *S.* × *curtilobum*, *S.* × *juzepczukii*, and *S. vernei* is conferred by Rx genes (Rx_{cha}, Rx_{cur}, Rx_{juz}, and Rx_{vrn}, respectively) that employ the same mechanism of interaction with the PVX coat protein as Rx, Rx_{acl}, and Rx_{adg}. Although *S. sucrense* clone OCH 11926.4 was resistant to both common PVX strains and PVX_{HB}, it was susceptible to the mutant isolates. We hypothesized that at least two viral determinants, one of which is not located in the coat protein coding region, interact with the *S. sucrense* resistance gene.

Additional keyword: potato resistance genes.

The first case of extreme resistance to potato virus X (PVX) was reported by Schultz and Raleigh (31) and Schultz et al. (30). This resistance, based on a single dominant gene designated Rx (7,18), was obtained from the Chilean potato cultivar Villaroela (*Solanum tuberosum* subsp. *tuberosum*). This gene was transferred to the USDA potato seedling 41956 and then incorporated into the North American potato cultivars Atlantic (35), Saco (2), and others (28). An additional gene, Rx_{adg}, was originally detected by Wiersema (36) in a progeny from *S. tuberosum* subsp. *andigena* CPC 1673 resistant to pathotypes Ro-1 and Ro-4 of the nematode *Globodera rostochiensis*. Extreme resistance to PVX also was found by Ross (27) in the wild species *S. acaule*. This gene, Rx_{acl}, considered by Cockerham (7) to be distinct from Rx_{adg}, also is inherited as a monogenic dominant character. Rx_{acl} was introduced into several European cultivars (28), among them the cultivar Bzura, and into the Argentine cultivar Serrana INTA via the back-cross hybrid MPI 44.1016/10. Ritter et al. (26) have confirmed the independence of Rx_{adg} and Rx_{acl} by locating loci *Rx1* and *Rx2* at different positions on a restriction fragment length polymorphism map of potato. An additional unidentified gene for extreme resistance to PVX was found in *S. vernei* cultivars resistant to the nematode *G. pallida* and introduced into some Dutch cultivars (28).

PVX strains have been classified into four groups (6,7) according to their reactions with the genes for localized hypersensitivity (Nb, Nx) and extreme resistance (Rx). Strain PVX_{HB}, found in 7% of Bolivian clones of *S. tuberosum* subsp. *andigena* (14,19), resembles the normal group 4 strains in overcoming genes Nb and Nx but differs from these in its ability to overcome the effects of Rx genes conferring extreme resistance to PVX. However, C. Chuquillanqui and L. F. Salazar (*unpublished results*) found extreme resistance to PVX_{HB} in a clone of the wild *S. sucrense* accession OCH 11926, and Brown et al. (3) suggested the involvement of a single dominant gene.

With few exceptions, the way in which the various types of resistance work at the biochemical or physiological levels is not well understood. Some efforts in recent years have been devoted to gaining knowledge about the genetic basis of resistance and the genetic system controlling the behavior of pathogens. Particular interest has been given to the phenomenon called "virulence": the ability of a particular virus strain to multiply or cause disease in individuals of the host species containing genes for resistance to that virus. Virulent isolates able to overcome single-gene resistance under normal conditions have been found for several viruses (1,19,28).

The specific case of PVX_{HB} has been studied extensively and several experiments have been conducted to localize the viral sequences affecting the interaction with Rx and to analyze the basis of virulence. Santa Cruz et al. (29) showed that the virulence determinants for the Rx gene are located in the 3′ portion of the viral genome downstream from the replicase gene. Studies with hybrid PVX genomes obtained by joining the 5′ part of the genome of a British group 3 nonbreaking isolate of PVX (PVX_{UK3}) and the 3′ part of the PVX_{HB} genome made it possible to narrow the region involved in the resistance-breaking activity of strain HB to the viral coat protein gene (15). Köhm et al. (16), using the knowledge that the coat protein gene affects the interaction of PVX and Rx, were able to analyze the basis of the virulence of HB. By inoculation of protoplasts with mutant derivatives of both strains HB and CP4, they showed that the virulence is the absence

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of avirulence rather than the production of a specific factor that suppresses the effects of Rx. They also were able to infer from frameshift mutation of the coat protein gene that the avirulence factor in HB is the coat protein rather than the coat protein gene.

Querci et al. (25) compared the nucleotide and amino acid sequences of PVX_{HB} and nonbreaking strains of PVX, PVX_{X3} (13), PVX_S (33), and PVX_{cp} (22), and highlighted the presence, within the coat protein amino acid sequence, of eight residues "unique" for strain HB. Computer-directed mutational analysis, performed by replacing each of these eight amino acids in the PVX_{HB} coat protein sequence with the corresponding ones from the PVX_{cp} sequence followed by secondary structure predictions, revealed that only two (121 and 226) of the eight residues considered determine variation in the predicted protein structure. These observations suggested the involvement of one (or both) of these amino acid residues in the resistance-breaking activity of the strain HB. At the same time, Goulden et al. (12) analyzed a series of hybrid and mutant isolates of PVX_{HB} and PVX_{CP4}, a group 4 isolate (14), and concluded that extreme resistance expressed in cultivar Cara, which carries the *Rx1* locus, is affected by amino acids 121 and 127 of the viral coat protein, with amino acid 121 representing the major determinant.

TABLE 1. Characteristics of the potato virus X (PVX) strains and mutant isolates used

Isolate	Resistance group[a]	Origin	Reference
PVX_{cp}	2	Peru	10
PVX_{CP4}	4	UK	14
PVX_{HB}	RB	Bolivia	19
PVX_{KH2}	UK3 + (5650–6432) HB = RB	...	15
$PVX_{CP4\text{-}KR}$	RB	...	12
$PVX_{HB\text{-}TK}$	Not RB	...	12

[a] Resistance groups according to Cockerham (6). RB = resistance-breaking.

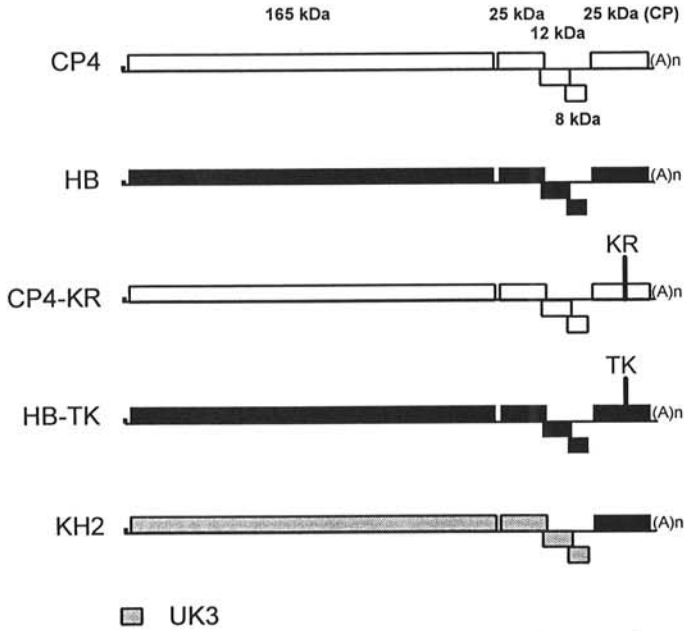

Fig. 1. Gene organization in potato virus X (PVX) indicating approximate size of the five open reading frames, and schematic representation of the recombinant and mutant isolates used. CP4 = wild-type PVX_{CP4} isolate from resistance group 4. HB = wild-type resistance-breaking strain PVX_{HB}. CP4-KR = coat protein mutant obtained in PVX_{CP4} cDNA by replacement of the codons of amino acids 121 and 127 with the corresponding ones from PVX_{HB}. HB-TK = coat protein mutant obtained in PVX_{HB} cDNA by replacement of the codons of amino acids 121 and 127 with the corresponding ones from PVX_{CP4}. KH2 = recombinant isolate from PVX_{UK3} in which the entire coat protein (CP) gene and 3′ noncoding sequence were replaced with the corresponding region from PVX_{HB}.

Wild tuber-bearing *Solanum* species possess a broad spectrum of resistance genes, but only a few have been analyzed and incorporated into the genome of commercial cultivars. Resistance currently used in breeding programs is still often limited to only a few sources. In addition, even the relationship among the widely used genes Rx, Rx_{acl}, and Rx_{adg} is essentially unknown.

Having demonstrated that the coat protein, specifically only a single amino acid at position 121, affects extreme resistance in cultivar Cara (*Rx1*), we analyzed a wider pool of Rx genes to find out whether different forms of Rx were affected by the same part of the viral coat protein. In this paper, we report the results obtained after inoculation of several commercial and native potato cultivars carrying Rx genes from different *Solanum* species with strains PVX_{HB}, PVX_{cp}, and PVX_{CP4} and with selected mutant forms of PVX containing specific, virulence-affecting mutations in the coat protein gene. The PVX constructs also were inoculated to the *S. sucrense* clone OCH 11926.4 carrying resistance to strain PVX_{HB} to determine whether this resistance was affected by sequence in the PVX coat protein.

MATERIALS AND METHODS

Virus strains and mutant isolates. Table 1 lists PVX strains and mutant isolates used in this work. Strains PVX_{cp} and PVX_{HB} were donated by C. Fribourg (Universidad Nacional Agraria La Molina, Lima, Peru) and maintained at the International Potato Center (CIP, Lima, Peru). PVX_{cp}, from strain group 2, was originally isolated from the high Andes of central-southern Peru (10; called PVX_C). PVX_{HB} is the resistance-breaking strain isolated in Bolivia by Moreira et al. (19). PVX_{CP4} is a strain group 4 isolate described by Jones (14).

The construction of the mutant isolates CP4-KR and HB-TK has been previously described (12). CP4-KR is a mutant derivative of PVX_{CP4} in which two point mutations have been introduced so amino acids 121 and 127 of the viral coat protein are lysine (K) and arginine (R), respectively, typical for strain HB coat protein, instead of threonine (T) and lysine (K), as normally found in PVX_{CP4}. HB-TK is a similarly produced mutant derivative of PVX_{HB} in which mutations have been introduced so amino acids 121 and 127 of the viral coat protein are T and K, as found in CP4, instead of K and R, respectively. KH2 is a hybrid PVX genome (K, PVX_{UK3}; H, PVX_{HB}) obtained by replacing the coat protein gene and 3′ noncoding sequence in the PVX_{UK3} genome with the homologous sequence from PVX_{HB} (15) (Fig. 1).

In all cases, stability of the mutations and identity of the progeny virus had been tested already. Kavanagh et al. (15) confirmed that hybrid KH2 had not been modified during passage on tobacco or potato and that the progeny virus was identical to the parental isolate by reverse transcription of the viral RNA recovered from infected potato plants and restriction enzyme or nucleotide sequence analysis of the polymerase chain reaction-amplified cDNA of the region between nucleotides 5110 and 6073. In a similar way, but amplifying the region between nucleotides 5617 and 6408, Goulden and Baulcombe (11) and Goulden et al. (12) confirmed the stability of the mutations in isolates CP4-KR and HB-TK.

Plant materials. The commercial and native potato cultivars and clones listed in Table 2 and used in experiment 1 were selected from the germ plasm collection held at the International Potato Center and propagated in vitro. In vitro plantlets were cut into single nodes and, after removal of large leaves, transferred to sterile flasks containing 25 ml of liquid Murashiga and Skoog (MS) medium (20) with 0.1 ppm of gibberellic acid and 2.5% sucrose. After growing for 3 weeks at 18 to 22°C with a 16-h photoperiod under 54 $\mu E\ m^{-2}\ s^{-1}$ light intensity per 24-h period, the plantlets were transferred to pots containing a compost mixture and maintained with high relative humidity for a few days (9) and finally transferred to a greenhouse for optimal leaf development prior to inoculations.

Wild *S. acaule* accessions (Table 2), supplied by C. Ochoa, used in experiment 2 were raised from botanical seeds. Plants of the wild *S. sucrense* accession OCH 11926 were grown from tubers that originated from the previously selected clone number 4 (OCH 11926.4) supplied by C. Lizárraga. Indicator plants were obtained from commercially available seeds.

Plant inoculations. All experiments were conducted in growth chambers at 18 to 22°C, with constant relative humidity (80%), and a 12-h photoperiod under 27 $\mu E\ m^{-2}\ s^{-1}$ light intensity per 24-h period. Wild strains and isolates of PVX (cp, CP4, and HB) were maintained in *Nicotiana glutinosa*. The mutant and hybrid viral cDNAs (CP4-KR, HB-TK, and KH2) were transcribed into infectious RNAs (4) and inoculated onto *N. glutinosa* and *N. clevelandii* as previously described (12,16).

Potato plants were manually inoculated with infective sap. Noninoculated leaves of infected *N. glutinosa* plants were homogenized in 50 mM sodium phosphate buffer (pH 7.0) at a rate of 1:10 (wt/vol) using a mortar and pestle. The inoculum was rubbed onto four leaves (previously dusted with 600-mesh Carborundum) per plant. Inoculated plants were maintained in a growth chamber under the conditions previously indicated and tested for PVX infection 3 and 4 weeks after inoculation.

Enzyme-linked immunosorbent assay (ELISA) and nucleic acid spot hybridization (NASH) tests. Virus infection was monitored by ELISA on nitrocellulose membranes (NCM-ELISA) and by NASH. NCM-ELISA was performed as described (17), but samples were macerated in Tris-buffered saline buffer (0.02 M Tris-HCl, 0.5 M NaCl, 0.01% NaN_3) containing 0.01 M DIECA and 0.01 M EDTA (5). The NASH test, performed according to Querci et al. (24) using the pX61 negative-sense ^{32}P-labeled RNA probe specific to PVX_{cp}, detected all PVX strains and isolates used in the experiments.

TABLE 2. Origin and resistance gene sources of potato cultivars, clones, and wild-species accessions used in the experiments

Potato[a]	CIP no.[b]	Origin	Gene	*Solanum* spp. resistance gene source
Atlantic Cv.	800827	U.S.	Rx	*S. tuberosum* subsp. *tuberosum* (USDA 41956)
Bzura Cv.	800953	Poland	Rx_{acl}	*S. acaule*
Carnera Native Cv.	703482	Peru	Rx_{adg}	*S. tuberosum* subsp. *andigena*
DTO-33 Clone	800174	CIP	rx	...
Huayro Native Cv.	703304	Peru	Rx_{cha}	*S.* × *chaucha* (natural hybrid)
LT-8 Clone	379706.27	CIP	Rx_{adg}	*S. tuberosum* subsp. *andigena*
Maria Huanca Cv.	279142.12	Peru	$Rx_{vrn?}$	*S. vernei* ?
Ugro Shiri Native Cv.	702937	Peru	Rx_{cur}	*S.* × *curtilobum* (natural hybrid)
Yagana INIA Cv.	720139	Chile	Rx_{vrn}	*S. vernei*
Yurac Kaipi Native Cv.	702078	Peru	Rx_{juz}	*S.* × *juzepczukii* (natural hybrid)
OCH 11818 WS	761027	Bolivia	Rx_{acl}	*S. acaule*
OCH 11823 WS	761286	Bolivia	Rx_{acl}	*S. acaule*
OCH 11825 WS	761288	Bolivia	Rx_{acl}	*S. acaule*
OCH 11889 WS	761330	Peru	Rx_{acl}	*S. acaule*
OCH 11890 WS	761331	Peru	Rx_{acl}	*S. acaule*
OCH 11912 WS	761347	Bolivia	Rx_{acl}	*S. acaule*
OCH 11983 WS	761379	Bolivia	Rx_{acl}	*S. acaule*
OCH 14391 WS	762032	Peru	rx	...
OCH 14392 WS	762033	Peru	rx	...
OCH 11926 WS	761351	Bolivia	?	*S. sucrense*

[a] Cv. = cultivar; WS = wild species.
[b] International Potato Center (CIP, Lima, Peru).

RESULTS

Five repetitions of each genotype were used for inoculation with each of the PVX strains or mutants listed in Table 1 and Figure 1. The results from experiment 1, summarized in Table 3, were confirmed in a second experiment conducted in a similar way. Isolates cp and CP4 behaved similarly on all potato genotypes. Both isolates were used in all experiments but for the sake of simplicity, only the results with the CP4 isolate are shown. Similarly, two HB isolates have been tested: HB-CIP (25) maintained at the International Potato Center, and a second isolate maintained at the Sainsbury Laboratory (Norwich, UK). Results reported for the HB strain mean similar results were obtained with both isolates. Differences in behavior or symptom production found between the two HB isolates are specifically indicated.

As expected, cp and CP4 isolates produced a systemic infection with mild mosaic only in the clone DTO-33 used as a susceptible (rx) control (Table 3), whereas strain HB also systemically infected all Rx-carrying genotypes tested. As indicated in Table 3, the recombinant clone KH2, originating from the group 3 PVX

TABLE 3. Infectivity of wild strains and mutant derivatives of potato virus X (PVX) tested on potato cultivars, clones, and wild species carrying extreme resistance genes, as assessed by nitrocellulose membrane–enzyme-linked immunosorbent assay and nucleic acid spot hybridization tests 3 and 4 weeks postinoculation

Exp.			PVX isolate[b,c]				
	Plant[a]	Gene	CP4	HB	CP4-KR	HB-TK	KH2
1							
	Atlantic	Rx	0/5	5/5	5/5	0/5	5/5
	Bzura	Rx_{acl}	0/4	3/3	4/4	0/5	3/3
	Carnera	Rx_{adg}	0/5	5/5	5/5	0/5	5/5
	DTO-33	rx	5/5	5/5	5/5	5/5	5/5
	Huayro	Rx_{cha}	0/5	5/5	5/5	0/5	5/5
	LT-8	Rx_{adg}	0/5	5/5	5/5	0/5	5/5
	Maria Huanca	$Rx_{vrn?}$	0/5	5/5	5/5	0/5	5/5
	Ugro Shiri	Rx_{cur}	0/5	5/5	5/5	0/5	5/5
	Yagana INIA	Rx_{vrn}	0/5	5/5	5/5	0/5	5/5
	Yurac Kaipi	Rx_{juz}	0/5	5/5	5/5	0/5	5/5
2							
	OCH 11818	Rx_{acl}	0/4	4/4	4/4	0/4	4/4
	OCH 11823	Rx_{acl}	0/4	4/4	4/4	0/4	4/4
	OCH 11825	Rx_{acl}	0/4	4/4	4/4	0/4	4/4
	OCH 11889	Rx_{acl}	0/4	4/4	4/4	0/4	4/4
	OCH 11890	Rx_{acl}	0/4	4/4	4/4	0/4	4/4
	OCH 11912	Rx_{acl}	0/4	4/4	4/4	0/4	4/4
	OCH 11983	Rx_{acl}	0/4	3/4	4/4	0/4	3/4
	OCH 14391	rx	3/3	1/3	1/2	2/3	3/3
	OCH 14392	rx	nt[d]	2/2	nt	nt	2/2
3							
	OCH 11926.4	?	0/7	0/7	4/7	nt	7/7

[a] Table 2 contains detailed plant information.
[b] Figure 1 contains detailed information on PVX isolates.
[c] Values are the number of systemically infected plants/total plants tested.
[d] nt = not tested.

isolate UK3, but carrying the PVX_{HB} coat protein gene (15), infected all genotypes tested. Goulden et al. (12) tested several mutant derivatives of PVX_{HB} and PVX_{CP4} and showed that a single feature in the coat protein affected virulence of HB on cultivar Cara (*Rx1*). These results indicated that only amino acid 121 of the coat protein is directly involved in the Rx-breaking activity of HB, whereas mutation at position 127 provides stability needed for normal accumulation of the virus (12). Accordingly, the mutant CP4-KR (mutated to have K and R in positions 121 and 127 of the viral coat protein, respectively, instead of T and K, as normally found in PVX_{CP4}) behaved on cultivar Cara as HB did. Conversely, the mutant derivative of HB, HB-TK, in which amino acids 121 and 127 of the viral coat protein are T and K, respectively, instead of K and R, behaved similarly to PVX_{CP4}. As shown in Table 3 and summarized in Figure 2, the behavior of these two mutant isolates, previously tested on cultivar Cara, was confirmed on all genotypes tested carrying resistance genes from different *Solanum* species. Although PVX_{HB} infection was symptomless in all genotypes tested, the mutant isolate CP4-KR induced a very mild mosaic in most genotypes and was most visible on cultivars Atlantic and Bzura. Genotypes infected with the recombinant clone KH2 developed a more defined and readily visible mosaic.

A selection of wild *S. acaule* accessions was included in an additional experiment (Table 3, experiment 2). Accessions OCH 11818, OCH 11823, OCH 11825, OCH 11912, and OCH 11983, collected in different locations in Bolivia, as well as Peruvian accessions OCH 11889 and OCH 11890, previously tested, were considered immune to PVX (L. F. Salazar, *unpublished results*). Peruvian accessions OCH 14391 and OCH 14392 were included as susceptible controls. Again, systemic infection was obtained in all accessions inoculated with strain HB as well as with the recombinant clone KH2 and the mutant isolate CP4-KR.

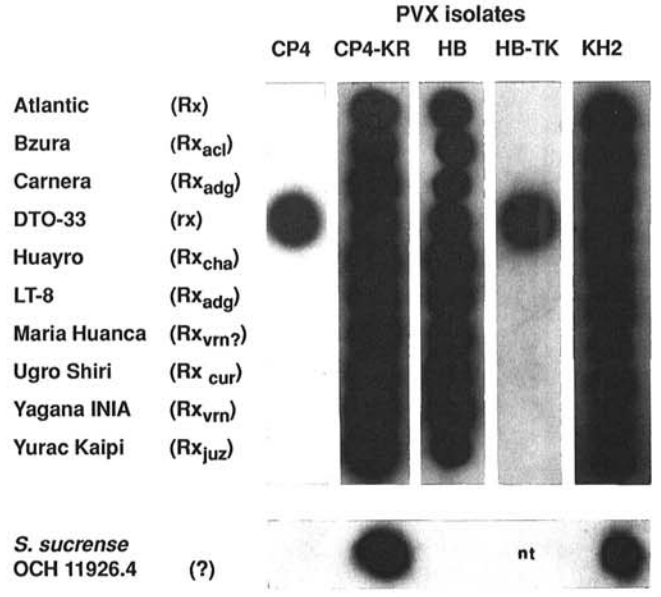

Fig. 2. Detection by nucleic acid spot hybridization of wild and mutant potato virus X (PVX) isolates in potato genotypes expressing extreme resistance to PVX. Leaf samples were taken 4 weeks postinoculation, spotted onto nitrocellulose membrane, and hybridized with ^{32}P-labeled RNA probe pX61 (24). CP4 = wild-type PVX_{CP4} isolate from resistance group 4. HB = wild-type resistance-breaking strain PVX_{HB}. CP4-KR = coat protein mutant obtained in PVX_{CP4} cDNA by replacement of the codons of amino acids 121 and 127 with the corresponding ones from PVX_{HB}. HB-TK = coat protein mutant obtained in PVX_{HB} cDNA by replacement of the codons of amino acids 121 and 127 with the corresponding ones from PVX_{CP4}. KH2 = recombinant isolate from PVX_{UK3} in which the entire coat protein gene and 3′ noncoding sequence have been replaced with the corresponding region from PVX_{HB}.

Isolates cp and CP4 infected only the susceptible accessions OCH 14391 and OCH 14392 and induced a mild mosaic in both. The mutant isolate HB-TK also infected only the accessions susceptible to CP4, but infection was symptomless. Although accessions immune to isolates cp and CP4 became systemically infected with the two isolates of the HB strain, they remained symptomless. The mutant isolate CP4-KR induced in all infected accessions a mild mosaic similar to the effects of CP4 on susceptible accessions. The hybrid clone KH2, which produces symptoms similar to PVX_{UK3} rather than PVX_{HB} on both tobacco and potato (15), produced a more severe mosaic in all infected accessions.

In addition, the *S. sucrense* clone OCH 11926.4, resistant to PVX_{HB}, was included in an additional experiment. To test each viral strain or isolate, seven plants were mechanically inoculated and maintained as indicated previously. Inoculated plants were assayed by NASH and NCM-ELISA tests 3 and 4 weeks postinoculation. As expected, no infection of *S. sucrense* was obtained with strains cp, CP4, and HB. Surprisingly, however, the mutant isolate CP4-KR and the recombinant clone KH2 produced systemic infection in 4:7 and 7:7 inoculated plants, respectively (Table 3, experiment 3). The presence of PVX particles in the noninoculated leaves of these plants also was confirmed by electron microscopy (data not shown). Furthermore, plants inoculated with the recombinant clone KH2 developed a necrotic response. Necrotic spots first appeared on inoculated leaves 10 days after inoculation (Fig. 3). Necrosis then spread to other parts of the plants, and general necrosis led to the death of all the plants approximately 4 weeks after inoculation. Two weeks after inoculation, no visible symptoms were present on the CP4-KR–in-

Fig. 3. Symptoms induced by the potato virus X recombinant isolate KH2 (15) on the *Solanum sucrense* clone OCH 11926.4. Necrotic spots first appeared on inoculated leaves about 10 days postinoculation. Necrosis then spread to other parts and led to the death of the plant.

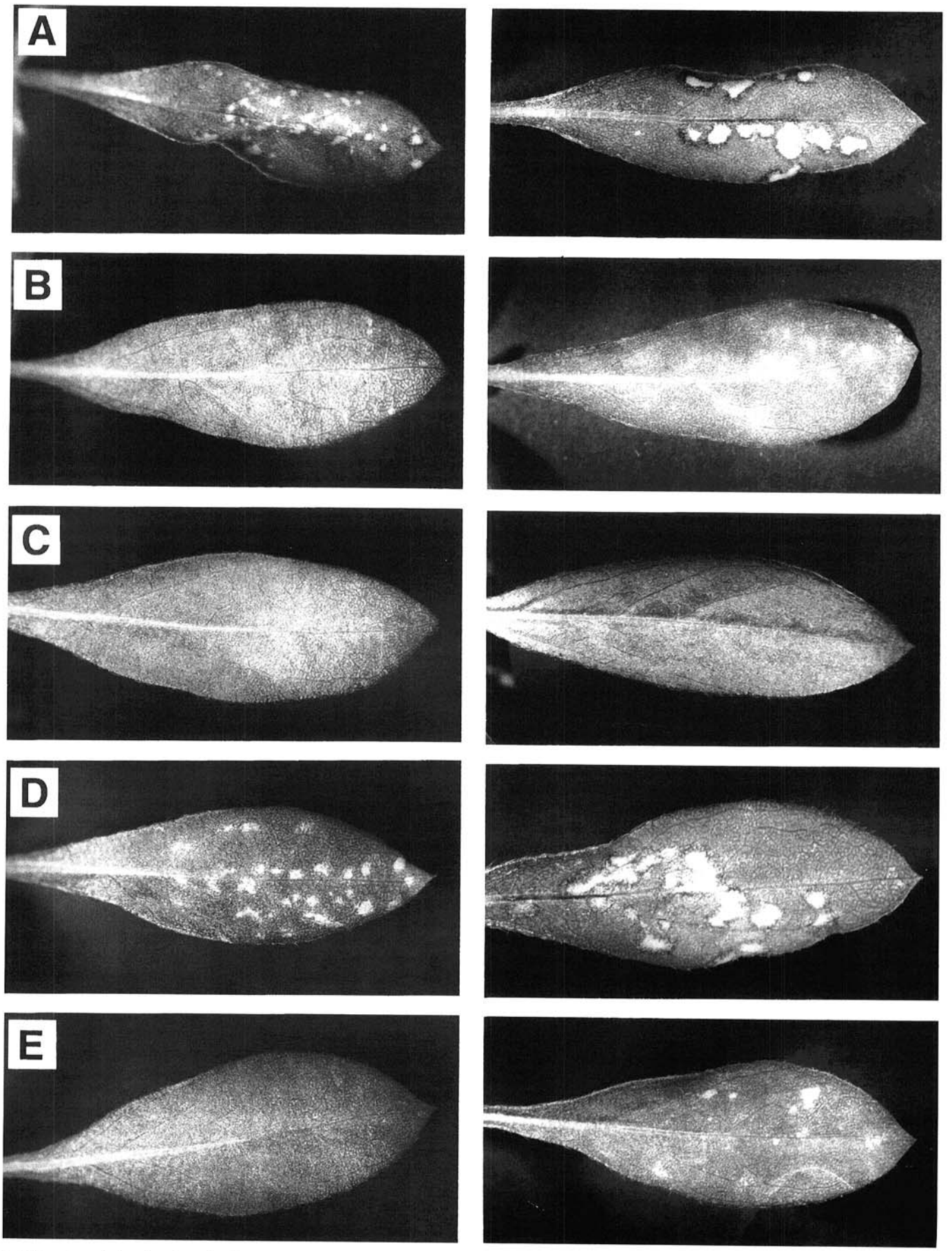

Fig. 4. Symptoms induced on inoculated leaves of *Gomphrena globosa* by wild and mutant isolates of potato virus X (PVX) 6 and 12 days postinoculation. **A,** PVX_{CP4}; **B,** $PVX_{CP4\text{-}KR}$; **C,** PVX_{HB}; **D,** $PVX_{HB\text{-}TK}$; **E,** PVX_{KH2}.

fected plants, but later they also developed general necrosis resulting in plant death. However, two of the four plants infected, in which PVX was present at a lower concentration, recovered and developed a mild mosaic in the young leaves produced.

The symptom expression of the PVX strains and mutants was analyzed on a broad range of indicator plants. Analysis of symptom production of the two mutant isolates CP4-KR and HB-TK showed that, except for *Gomphrena globosa*, all indicator plants tested developed symptoms indistinguishable from the original parental strains from which the mutants were derived. Indeed, symptom production determinants for almost all indicator plants were not affected by the mutations. On the contrary, *G. globosa*, which is the only indicator plant available to clearly distinguish PVX_{HB} from all other PVX strains, seems to be susceptible to the mutations induced in the coat protein gene. Whereas isolate CP4 induced well-defined necrotic spots on *G. globosa* 5 to 6 days postinoculation, the mutant CP4-KR did not. Similarly, mutant HB-TK behaved on this host as a common PVX strain, producing local necrotic spots with well-defined red rings, whereas the wild-strain HB did not produce any symptoms.

The involvement of noncoat protein determinants in symptom development on most indicator plants and of the coat protein gene in the case of *G. globosa*, also has been confirmed with KH2. This recombinant isolate induced severe symptoms similar to CP4 in all indicator plants tested, but behaved on *G. globosa* like HB in that it did not produce local lesions. However, some necrotic spots, not well defined and without typical red rings, appeared approximately 2 weeks after inoculation on *G. globosa* leaves inoculated with the mutant isolates CP4-KR and KH2 (Fig. 4).

DISCUSSION

After introduction into a host plant, viruses may activate resistance genes that initiate different types of resistance reactions. Despite considerable attention in recent years, the primary products of resistance genes, and the way in which these products interact with viruses, have remained essentially unknown.

The importance of the viral coat protein in resistance and symptom expression has been shown for several virus-plant combinations (8,21,23,32). Kavanagh et al. (15) demonstrated that in the case of PVX the coat protein gene plays an important role in viral pathogenicity and, by analyzing different hybrid PVX genomes, including elements of PVX_{UK3} and PVX_{HB}, located the resistance-breaking properties of PVX_{HB} in the coat protein gene. In an attempt to establish more precisely the exact location of the determinants in the PVX coat protein sequence responsible for the ability of HB to overcome Rx resistance, Querci et al. (25) compared the nucleotide and amino acid sequences of PVX_{HB} with those reported for three nonresistance-breaking strains of PVX: PVX_{X3} (13), PVX_S (33), and PVX_{cp} (22). This comparison highlighted a high degree of similarity in the coat protein sequences with only a few differences limited to a small number of residues. Of the eight amino acid residues unique to the HB coat protein sequence, only two determined variation in the coat protein structure, i.e., K_{121} and A_{226}. In that work (25), it was assumed that one or both of these amino acid residues was responsible for the biological differences between PVX_{HB} (Rx-breaking) and the other strains (nonbreaking).

Using a series of hybrid and mutant isolates of PVX_{HB} and PVX_{CP4}, Goulden et al. (12) determined that elicitation of resistance was affected only by amino acids 121 and 127 of the viral coat protein. PVX_{HB} and hybrid or mutant isolates with lysine and arginine at positions 121 and 127 were able to overcome resistance expressed on cultivar Cara (*Rx1*), whereas those with threonine and arginine were resistance-sensitive. That work (12), in accordance with the prediction of Querci et al. (25), also pointed out that position 121 is the major determinant in resistance-breaking activity, whereas the importance of residue 127 is suggested

TABLE 4. Symptoms induced on indicator plants by potato virus X (PVX) strains CP4 and HB and mutant isolates CP4-KR, HB-TK, and KH2

Indicator plant	PVX isolate[a]					
	CP4	CP4-KR	HB	HB (CIP)	HB-TK	KH2
Amaranthus caudatus	LCS	LCS	–	–	–	LCS
Amaranthus edulis	–	LCS	–	–	–	LCS
Gomphrena globosa	LNS + red rings	Late LNS and no red rings, SI	–	–	LNS + red rings	Late LNS and no red rings
Chenopodium amaranticolor	LCS, Few SCS	LCS, SCS	Mild LCS	LCS, SCS	Mild LCS, Few SCS	LCS, SCS
Chenopodium murale	LNS	LNS	–	–	-	LNS
Chenopodium quinoa	LCS, SCS	LCS, SCS	LCS	LCS	LCS, SCS	LCS, SCS
Helianthus annuus	MM, LCR	LCR	–	–	–	–
Cucumis sativus	–	–	–	–	–	–
Datura metel	SM, SNS, ND	SM, SNS, ND	Very MM, LNS, SVC	MM, LNS, SVC	Very MM, LNS	SM, SNS, ND
Datura stramonium	SM, SNS, SNR, Df	SM, SNS, SNR, Df	MM	MM	MM	SM, SNS, Df
Lycopersicon esculentum cv. Rutgers	SM, SVC	SM, SVC	–	MM	MM	SM
Nicandra physaloides	SM, SNR	SM, SNR	Very MM	Very MM	Very MM	SM, Few SNS
Nicotiana benthamiana	SM, AN, SNS, Df	SM, AN, SNS, Df	MM, SCS	MM, SCS	MM, SCS	SM, AN, SNS, Df
Nicotiana clevelandii	SM, SVN	SM, SVN	MM	MM	MM	SM
Nicotiana debneyi	SCR	SM, SCR, SVN	MM	MM, SVN	MM	SM, SVN
Nicotiana glutinosa	SM, SVC, SNS	SM, SVC, SNS	Very MM	MM	Very MM	SM, SNS
Nicotiana occidentalis	SM, SVN	SM, SVN	SCR	MM	MM	SM, SVN
Nicotiana rustica	SCR	SCR	Mild SCR	Mild SCR	Mild SCR	SM, SCR
Nicotiana tabacum cv. Samsun	SNS, SNR, SCS	SNS, SNR, SCS	SS	Mild SCR	SS	SNS, SNR
Physalis floridana	SM, SVC, SNR	SM, SVC, SNS	SS	Very MM	SS	SM, SVC, SNS
Coriandrum sativum	SS	SS	–	–	SS	–
Phaseolus vulgaris	–	–	–	–	–	–

[a] LCS = local chlorotic spots; LCR = local chlorotic rings or ringspots; LNS = local necrotic spots; MM = mild mosaic; SM = severe mosaic; SCS = systemic chlorotic spotting; SNS = systemic necrotic spots; SNR = systemic necrotic rings or ringspots; SVC = systemic vein clearing; SS = symptomless systemic infection; SI = systemic infection; Df = systemic leaf deformation; ND = necrotic defoliation; SVN = systemic vein necrosis; AN = apical necrosis; – = no symptoms, no systemic infection.

to be linked to the stability of the mutants. In this work, we analyzed the recombinant clone KH2 (15) and two mutant isolates, CP4-KR and HB-TK (12), and tested their ability to overcome the effects of Rx genes originating from different *Solanum* species. From the results obtained, we concluded that the resistance-breaking capacity determined by the two amino acid residues in positions 121 and 127 within the HB coat protein affects all Rx genes tested (Rx, Rx_{acl}, Rx_{adg}, Rx_{cha}, Rx_{cur}, Rx_{juz}, and Rx_{vrn}). Hence, the mechanism of recognition (of the PVX coat protein) seems to be the same for all the genes considered (Table 3; Fig. 2).

The various primitive cultivated and wild potato species represent diverse gene pools and possess great variability for many traits. Moreover, they are and have been indispensable sources for resistance to pathogens (28). Genes from several wild potato species have been incorporated into the genome of many cultivars, even if at present only a small portion of the valuable genes found (or present) in wild species has been used. Most breeding for resistance has been done at an empirical level, without a detailed knowledge of the genetic basis. In the specific case of PVX, the exact relationship among the different Rx genes incorporated into various potato cultivars was not known.

We can identify extreme resistance found in *S.* × *chaucha*, *S.* × *curtilobum*, *S.* × *juzepczukii*, and *S. vernei* as conferred by Rx genes (Rx_{cha}, Rx_{cur}, Rx_{juz}, and Rx_{vrn}, respectively) that interact with the PVX coat protein in the same manner Rx (USDA 41956), Rx_{acl}, and Rx_{adg} do.

Symptoms produced on *N. glutinosa* and *N. clevelandii* by the recombinant clone KH2 and the mutant isolates CP4-KR and HB-TK resembled symptoms produced by the PVX strains from which they were prepared (UK3, CP4, and HB, respectively). The substitutions or alterations induced within the coat protein gene did not affect symptom production on these hosts. For that reason, we analyzed a broad range of indicator plants and compared the symptoms produced by the mutant isolates and by the wild-type PVX strains (Table 4). In all cases, except for *G. globosa*, we confirmed that alterations produced in the coat protein gene did not affect or alter symptom production. *G. globosa* is the only indicator plant currently available that clearly distinguishes PVX_{HB} from all other strains of PVX. As indicated by Goulden et al. (12), the coat protein and, specifically, residues 121 and 127 of the coat protein also are involved in this characteristic of HB. Indeed, the ability to overcome resistance is associated with a lack of local lesion production on inoculated leaves of *G. globosa* (Fig. 4).

All PVX strains and mutant isolates KH2 and CP4-KR were tested on the *S. sucrense* accession OCH 11926 (clone OCH 11926.4) reported to be immune to PVX_{HB}. In addition to the results obtained and summarized in Table 3 and Figure 2, an additional experiment, conducted to confirm the results, also included the mutant isolate HB-TK. All previous results were confirmed. Furthermore, HB-TK also infected OCH 11926.4 plants (data not shown). HB-TK–infected plants produced very small necrotic lesions on inoculated leaves and, again, plant decay and early plant death. The findings that both the recombinant clone KH2 and the mutant isolates CP4-KR and HB-TK, but not the parental isolates, are able to systemically infect this accession open up a new question as to what kind of resistance mechanism is involved. Homologous combinations of coat protein genes and upstream sequences result in resistance (Table 5). However, when the coat protein gene of HB is placed in a CP4/UK3 context, the resistance is broken. A CP4 coat protein gene placed in an HB context also led to resistance breakage. Although we cannot precisely define the mechanism of action of the resistance gene found in the *S. sucrense* clone OCH 11926.4 at this time, a first interpretation of the results suggests that in this case resistance or susceptibility is not determined solely by an interaction with a single determinant in the coat protein, but by two determinants, the second of which maps outside the coat protein and the 3′ noncoding region.

TABLE 5. Reaction of *Solanum sucrense* clone OCH 11926.4 to wild strains and mutant isolates of potato virus X (PVX)

PVX	5′ region characteristic	Coat protein		OCH 11926.4 reaction[a]
		Origin	aa 121	
Strain				
cp	cp	cp	T	R
CP4	CP4	CP4	T	R
HB	HB	HB	K	R
UK3	UK3	UK3	T	nt
Mutant				
CP4-KR	CP4	CP4	K	s
KH2	UK3	HB	K	s
HB-TK	HB	HB	T	s

[a] R = resistance; s = susceptible; nt = not tested.

It also is important to stress the high value of the resistance carried by this accession. OCH 11926 carries, in addition to immunity to PVX, other useful characteristics of resistance to several important viruses that infect potato. In inoculation experiments (L. F. Salazar, *unpublished results*), this *S. sucrense* accession also showed resistance to potato leafroll virus, hypersensitivity to potato virus Y, resistance to potato viruses T and S, and immunity to Andean potato mottle virus. Recently, Tozzini et al. (34) reported an additional source of resistance to PVX_{HB} found in *S. commersonii* and tentatively defined this new type of resistance as IRx_{com}. At present, it is not known if resistance induced by this IRx gene acts similarly to that found in the *S. sucrense* accession OCH 11926. Knowledge of the genetic basis of this resistance and of the complementary genetic system controlling the behavior of viral pathogens, and of their interaction, could provide clues to understand the underlying mechanism. Further studies are needed to specifically characterize the novel *S. sucrense* gene. The use of specific mutant isolates could help to better understand the mechanism of resistance involved.

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