Transgenic Potato Virus Y Resistance in Potato: Evidence for an RNA-Mediated Cellular Response

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ABSTRACT

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This study was undertaken to assess the potential of transgenic RNA-mediated virus resistance in potato. Transgenes that expressed various versions of the potato virus Y (PVY) coat protein (CP) open reading frame (ORF) were constructed and expressed in potato. Plants highly resistant to PVY infection could be generated by expressing transgenes from which either mRNA and CP or only an untranslatable mRNA were made. An inverse correlation between transgene transcript accumulation and virus resistance was often noted when translatable or untranslatable versions of the PVY CP ORF were expressed. The data suggest that virus resistance is likely mediated by a host cell response. A dominant negative phenotype resulting from the transgene product competing with the viral-encoded product is not supported by the data.

The demonstration of engineered virus resistance has become commonplace; however, the mechanisms responsible for establishing the resistant state remain ill-defined (reviewed in 1,2,31). The concept of pathogen-derived resistance is firmly rooted in these studies (24). The central dogma of this theory is that the synthesis of a viral protein or viral RNA at the inappropriate time, at an incorrect level, or in a dysfunctional form will interfere with normal virus/host or viral/viral interactions and result in attenuated or aborted virus replication.

There are a number of reports that describe the establishment of potyvirus resistance in transgenic plants (7,11,12,14,15,19,20,25, 27,28). An analysis of the biochemical data from these transgenic plant studies reveals that highly resistant lines frequently, and unexpectedly, accumulate low levels of the transgene product. This is inconsistent with the action of a dominant negative phenotype (10) and the theory of pathogen-derived resistance (24).

It has been shown that transgenes expressing untranslatable sense RNAs can confer an extremely effective level of potyvirus resistance in tobacco. This resistance, coined RNA-mediated resistance, is effective in laboratory (16), greenhouse (5,15,25) and field settings (30). The present study was undertaken to assess this resistance strategy in two commercial cultivars of potato. Transgenic 'Russet Burbank' and 'Russet Norkotah' lines were established that displayed a high level of resistance to potato virus Y (PVY), the type member of the potyvirus family (13,21). Plants displaying resistance had biochemical features characteristic of RNA-mediated resistance (5,25). These results are discussed in the context of previous transgenic plant virus resistance studies.

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MATERIALS AND METHODS

Virus isolates and origin of potato tissue. The PVY-nn isolate was obtained from G. Gooding, North Carolina State University, Raleigh. The PVY-A26 isolate was isolated from infected potatoes at the Oregon State University Research and Education Complex, Hermiston. The PVY-DC isolate was acquired from D. Corsini, USDA/ARS Aberdeen, ID, and is a representative PVY isolate found in potatoes in eastern Idaho. Viruses were maintained in *Nicotiana tabacum* L. 'Burley 21' (B21). These three isolates produced mild to no visible symptoms when potatoes were infected and grown under our greenhouse conditions. Infection of potato tissue was determined using an enzyme-linked immunosorbent assay (ELISA) and/or back-inoculation of infected potato leaf tissue to B21. The PVY-nn isolate caused veinal necrosis on B21, whereas PVY-A26 and PVY-DC induced mild mosaic symptoms on tobacco and did not cause necrosis. Potato cultivars Russet Burbank (RB) and Russet Norkotah (RN) are available commercially.

Construction of transgenes. Construction of 35S, coat protein (CP), RC-1, and antisense (AS) transgene constructs was as described in Smith et al. (25). RC-4 was constructed using the same mutagenesis strategy, except that three additional stop codons were introduced after the initiation codon. Pertinent features of the transgene constructs and the expected plant transgene products are presented in Figure 1.

Generation of transgenic potatoes and maintenance in tissue culture. *Agrobacterium*-mediated stem and leaf disk transformations were performed as adapted from De Block (4). The transformation procedure was carried out with the following exceptions: De Block's S1 medium was replaced with a long-term shoot-regeneration medium containing 1.03 g of Gamborg's B-5 basal salts per liter (Sigma Chemical Co., St. Louis), 2.86 g of MS salts per liter (Sigma, M-5524), 37.0 μM glycine, 4.0 μM nicotinic acid, 2.0 μM pyridoxine monohydrochloride, 1.0 μM thiamine hydrochloride, 0.6 μM folic acid, 0.2 μM *d*-biotin, 0.5 mM myo-

inositol, 0.1 μM naphthaleneacetic acid, 87.0 mM sucrose, and 0.8% purified agar (Sigma, A-7921). This solution was adjusted to pH 5.6, and 50 mg of kanamycin and 250 mg of ticillin per liter were added prior to aliquoting. The S2 medium contained 50 mg of kanamycin and 1.0 g of ticillin per liter rather than De Block's carbenicillin or cefotaxime. Finally, transformed leaf disks and internodal stem pieces were incubated on S2 medium for 3 days. Established transgenic potato lines were maintained in tissue culture on the long-term medium; fresh shoots were transferred approximately every 3 to 4 weeks.

Whole plant inoculation experiments. Virus inoculum was prepared as a 1:10 dilution (wt/vol) of virus-infected tobacco leaf tissue in deionized and distilled water. Plant leaves were lightly dusted with Carborundum, and 50 to 100 μl of virus inoculum was applied with a cotton swab. Plants were typically assayed 21 to 28 days later. Plant/virus interactions were grouped into three broad categories: susceptible (Sus.), a systemic virus replication followed inoculation, and virus progeny CP and virions were detected; resistant (Res.), PVY was able to replicate in the inoculated leaf, but PVY was not detected in other leaves; highly resistant (H. Res.), there were no detectable levels of virus replication in inoculated or systemic leaf tissue.

Protein analysis. Anti-PVY CP polyclonal primary antibodies (25) were used at a 1:1,000 dilution to detect transgene-derived PVY CP. The Western gel blot analysis procedure described for tobacco etch virus (TEV) CP (15) was used. ELISA was carried out using the protocol of Converse and Martin (3).

RNA analysis. Total RNA was isolated from transgenic plants by LiCl precipitation (29). Denaturing RNA gels, RNA gel blotting, and synthesis (via a SP6/T7-based cell-free transcription system) and hybridization of strand-specific ^{32}P-labeled RNA probes were performed as described previously (25).

DNA analysis. Plant genomic DNA was extracted as described by Rogers and Bendich (22). Genomic DNA was digested with a restriction enzyme that cut the DNA at a single site within the transferred DNA. Enzymes were used according to the manufacturer's recommendations. Southern blotting procedures were performed as described by Sambrook et al. (23). Southern gel blots were hybridized with α-^{32}P dCTP-labeled PVY CP DNA fragments. CP DNA probes were synthesized by the random prime method of Feinberg and Vogelstein (9) using a random primer extension labeling kit (Du Pont Co., Wilmington, DE).

RESULTS

The transgenes used in this study are presented in Figure 1. The PVY CP transgene expressed a mRNA that was translated into PVY CP molecules. The amino acid sequence of the transgenic PVY CP molecule was identical to CP derived from the viral genome, except that it contained the N-terminal sequence methionine-glycine instead of the naturally occurring glycine-asparagine. RC-1 and RC-4 transgenes expressed a mRNA that had been rendered untranslatable by limited nucleotide changes introduced via site-directed mutagenesis. One (RC-1) or four (RC-4) nonsense codons were inserted proximal to the initiation codon of the CP open reading frame (ORF). The nucleotide sequence at the 5′ end of the transgenic PVY CP ORF and mutations introduced to form stop codons are presented in Figure 1. PVY antisense (AS) transgenes also expressed an untranslatable RNA; however, the PVY CP ORF was inserted in the reverse orientation downstream of the constitutive cauliflower mosaic virus (CaMV) 35S promoter.

Approximately 200 transgenic potato lines were generated; 69 were selected as representative and examined in detail. Table 1 summarizes the results of these studies. Transgenic potato lines were designated as follows: Russet Burbank containing CP transgene, RCP; Russet Burbank containing RC-1 transgene, R1; Russet Burbank containing RC-4 transgene, R4; Russet Burbank containing AS transgene, RA; and Russet Burbank containing the CaMV 35S sequences, RS. Russet Norkotah lines followed a similar nomenclature, except the R was replaced with an N (i.e., RCP to NCP). The numerical identifier after the transgenic line nomenclature designates a particular transgenic line.

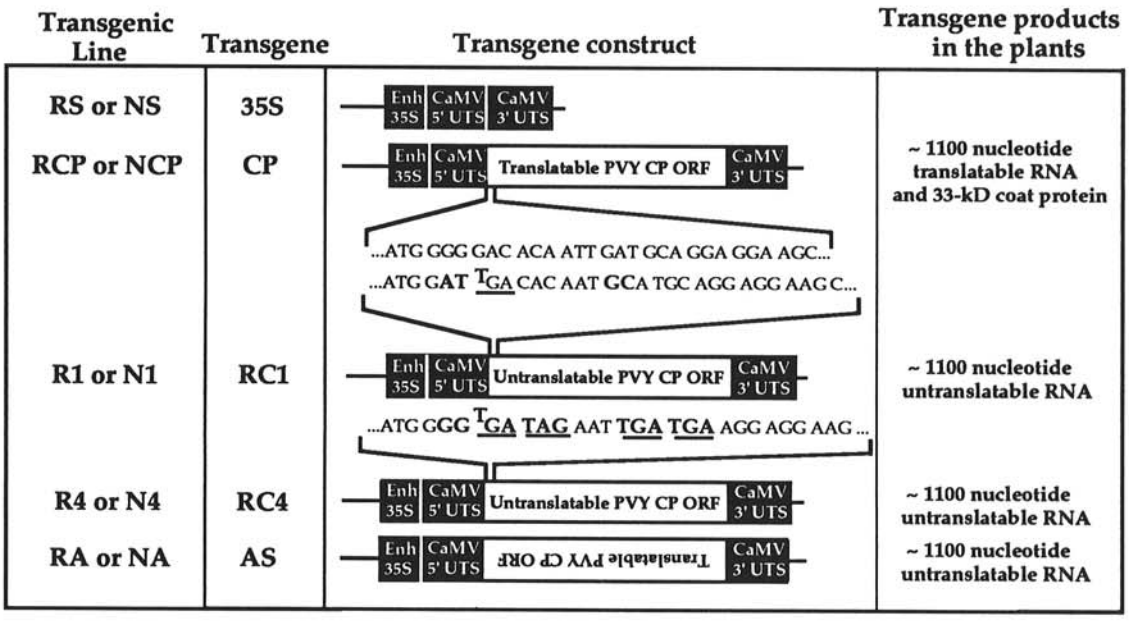

Fig. 1. Schematic representation of potato virus Y (PVY) transgenes. The five transgenes constructed and inserted into potato are presented along with their nomenclature and expected products. The first letter of the transgenic line nomenclature indicates germ plasm transformed ('Russet Burbank' [R] or 'Russet Norkotah' [N]). Cauliflower mosaic virus (CaMV) sequences used in the gene constructs are presented as filled-in boxes and represent the enhanced 35S DNA-dependent, RNA-polymerase promoter sequence (Enh 35S) and regions that make up the 5′ and 3′ untranslated region of the 35S mRNA. PVY coat protein (CP) sequences are represented by the labeled white boxes. The sequence comprising the first 30 nt at the 5′ end of the PVY CP open reading frame (ORF) is presented below the CP transgene. The corresponding region of the RC-1 transgene is presented below for comparison. Nucleotide changes from the translatable sequence are presented in bold, and the resulting nonsense codon is underlined. The RC-4 transgene mutations and four nonsense codons are presented above the RC-4 transgene. The antisense (AS) RNA-producing transgene is presented at the bottom of the figure and represents the translatable PVY CP ORF inserted in the incorrect orientation. The products expected from the transgenes are described on the right.

TABLE 1. Characteristics of transgenic potatoes with potato virus Y (PVY) transgenes

Line name[a]	PVY resistance phenotype[b]	Transgene RNA level[c]	PVY CP level[d]	Number of DNA bands[e]
R. Burbank	Sus.	ND	ND	ND
R. Norkotah	Sus.	ND	ND	ND
N1 2-022	Sus.	High		1
N1 2-023	Sus.	Medium		3
N1 2-024	H. Res.	Low		3/4**
N1 2-033	Sus.	High		2
N4 2-042	Res.	Low		4/5
N4 2-043	Sus.	High		2*
N4 2-044	Sus.	High		2
N4 2-045	Sus.	NT		1
N4 3-164	Res.	High		2
N4 3-176	Sus.	Medium		2
N4 3-191	Res.	Low		1
N4 3-192	Res.	Low		2*
N4 3-206	Res.	High		2
N4 3-210	H. Res.	Low		4*
N4 3-212	Sus.	Medium		2
N4 3-214	H. Res.	Low		5*
NA 1-004	Sus.	ND		1
NA 2-010	Sus.	Medium		1
NA 3-079	Sus.	Low		2
NA 3-097	Sus.	Medium		5/6**
NCP 2-018	Sus.	Medium	Medium	1
NCP 2-019	H. Res.	Low	ND	3
NCP 2-020	H. Res.	Low	Low	3
NCP 3-126	Sus.	Medium	Medium	2
NCP 3-128	Sus.	High	Medium	2
NCP 3-132	H. Res.	Low	ND	2
NCP 3-134	Sus.	Medium	Medium	2*
NCP 3-136	H. Res.	Low	ND	5/6*
NCP 3-138	H. Res.	Low	ND	1*
NCP 3-144	H. Res.	Low	Low	3**
NCP 3-152	Sus.	Low	Medium	NT
NCP 3-155	H. Res.	Low	ND	2
NCP 3-156	H. Res.	Low	ND	5/7
NS 2-053	Sus.	ND	ND	ND
NS 2-056	Sus.	ND		ND
R4 0-006	Sus.	Medium		2
R4 0-007	H. Res.	Low		5/7**
R4 0-009	Res.	Medium		3
R4 1-001	H. Res.	Medium		5*
R4 1-002	Sus.	Medium		2
R4 1-003	Res.	Medium		3
R4 2-035	Sus.	High		2
R4 2-037	Sus.	NT		2
R4 3-162	Sus.	Low		1
R4 3-174	H. Res.	Low		6*
R4 5-389	Sus.	NT		2
R4 6-455	H. Res.	Low		3*
R4 6-456	H. Res.	Low		3
R4 7-699	Res.	Low		5
RA 2-002	H. Res.	Medium		3*
RA 2-006	Sus.	High		7
RA 3-066	Sus.	Low		4
RA 3-077	Sus.	ND		3
RCP 2-011	Sus.	High	Medium	1
RCP 5-353	Res.	Low	High	2/3
RCP 5-361	Sus.	Medium	Low	2
RCP 6-428	H. Res.	Low	Low	2**
RCP 6-432	Sus.	High	Medium	1
RCP 6-510	H. Res.	Low	ND	4/5
RS 0-001	Sus.	ND		ND
RS 0-011	Sus.	ND		ND

[a] Transgenic plant nomenclature is described in text and presented in Figure 1. R. = Russet.

[b] The PVY resistance response was classified as: susceptible (Sus.), a systemic virus replication followed inoculation and virus progeny coat protein (CP) and virions were detected; resistant (Res.), PVY was able to replicate in the inoculated leaf but was not detected in other leaves; highly resistant (H. Res.), no evidence of virus replication in inoculated or systemic leaf tissue.

[c] The steady state level of sense and antisense RNA transcripts derived from various PVY CP open reading frame (ORF)-related transgenes is presented. Representative levels can be viewed in Figure 2A (lanes 5 and 14 [high], lanes 3, 12, and 18 through 20 [medium], and lanes 6 through 11 [low]). NT, not tested; ND, not detected.

[d] The steady state level of PVY CP derived from the PVY CP transgene was estimated by Western gel blot analyses. ND, not detected; low, less than 1 ng of PVY CP per 2.0 mg of leaf tissue; medium, between 1 and 10 ng of PVY CP per 2.0 mg of leaf tissue; high, over 10 ng of PVY CP per 2.0 mg of leaf tissue.

[e] Estimate of the transgene copy number. DNA from the various lines was isolated and digested with the restriction enzyme *Eco*RI. The DNA was separated by electrophoresis in agarose gels and blotted onto nylon. The blot was hybridized with ^{32}P-labeled PVY CP ORF-specific sequences. The number of bands detected is presented. One asterisk after the number indicates a band had a stronger hybridization signal than that of other bands. Two asterisks indicates two bands with an intense signal.

Resistance responses. Three responses were noted when transgenic potatoes were inoculated with PVY isolates. Many lines were susceptible (Sus.; Table 1) to PVY. Progeny virus CP could be detected by ELISA, and the presence of infectious progeny virus in systemically infected leaf tissue was demonstrated in back-inoculation studies to *N. tabacum* cv. Burley 21 (B21). A second response noted for a number of lines was the ability to establish PVY infection in inoculated leaves but failure to detect virus in other leaves as expected in a systemic infection. This sort of resistance response (Res.; Table 1) was noted only in a limited number of lines (R4 and N4) expressing the RC-4 transgene. The third type of response was a highly resistant response (H. Res.; Table 1) in which PVY was never detected in inoculated plants by ELISA or in back-inoculation studies to B21.

In general, highly resistant lines were observed for each group of transgenic plants, with the exception of lines NS, RS, and NA. These results are summarized in Table 1 and represent the compilation of 12 greenhouse experiments conducted over an 18-month period. No differences were noted between different PVY isolates tested with one exception. PVY-nn infection of any potato line consistently resulted in ELISA readings that were 25 to 50% of the values obtained with the other two PVY isolates. Not every line was examined in each experiment, but each line was assessed on at least 4 occasions.

Biochemical characteristics. The different responses to challenge-inoculation with PVY prompted us to ask whether there were molecular genetic or biochemical features that correlated with the different phenotypes. The steady state level of the transgene transcript was determined for all lines. Total RNA was extracted, separated by electrophoresis in a denaturing agarose gel, blotted to nitrocellulose, and hybridized with a ^{32}P-labeled riboprobe specific for the PVY transgene sequence. The expected transgene transcript was observed in all lines, except RS and NS. (These transgene-derived transcripts likely were too small to be detected in the experimental protocol.) Also noted with lines RCP, NCP, N1, N4, and R4 was a general, but not absolute, inverse relationship between the amount of transcript detected in Northern blot analysis and the highly resistant phenotype. This can be observed in the RNA gel blot presented in Figure 2A. Lines that accumulated a high level of the transgene transcript always possessed a PVY-susceptible phenotype; lines that accumulated low to barely detectable levels of the transgene transcript often displayed a resistant phenotype. We have arbitrarily classified RNA steady state levels as high (Fig. 2A, lanes 5 and 14), medium (Fig. 2A, lanes 3, 12, and 18 through 20), and low (Fig. 2A, lanes 6 through 11) and report these levels in Table 1.

A number of NA and RA lines expressing an AS version of the PVY CP ORF also were analyzed. Most of these lines possessed a PVY-susceptible phenotype; however, one RA line displayed a highly resistant phenotype. This line also was notable in RNA gel blot analysis because it possessed an additional higher molecular-weight band that hybridized with the PVY AS-specific riboprobe (Fig. 2B, lane 7).

RCP and NCP also were examined for steady state levels of PVY CP. ELISA and Western gel blots were performed using a polyclonal antibody to a glutathione S-transferase-PVY CP fusion protein generated in *Escherichia coli*. The CP steady state levels were estimated from Western gel blots (Fig. 3). Highly resistant NCP and RCP lines had low to undetectable steady state levels of the transgene-derived PVY CP. Susceptible lines often had medium levels of the PVY CP, although a few lines exhibited low levels of transgene-derived PVY CP. These correlations were consistent with the RNA steady state level observations above.

Most of the transgenic lines were examined by Southern gel blot analyses to assess the number of transgenes present. Genomic DNA was purified, digested with the restriction enzyme *Eco*RI, and fragments were separated by electrophoresis in agarose gels. The DNA was transferred to nylon and hybridized with randomly primed ^{32}P-

labeled DNA fragments specific for the PVY CP transgene. The results of one such analysis are presented in Figure 4.

Three observations were made from this study. First, multiple bands hybridizing to PVY sequences were observed frequently, ranging from two to a high of seven bands. (In some cases, a single band likely containing multiple transgenes, based on intensity of the hybridization signal, was detected.) Second, only one highly resistant line (NCP 3-138) possessed a genomic pattern of one band; the remaining resistant lines had two or more bands. However, the intensity of this band suggested more than one transgene copy could be present (Fig. 4, lane 43). Finally, most of the susceptible lines had one or two hybridizing bands; however, multiple bands were observed with some susceptible lines. These observations are summarized in Table 1.

DISCUSSION

There have been a number of reports detailing the generation of PVY-resistant tobacco and potato plants (7,8,12,14,25,26,27). Most of these studies have expressed transgenes that contain a PVY CP sequence; however, other PVY sequences have been expressed (20,28). A clear understanding of the underlying mechanism(s) responsible for the resistance phenotype is lacking, although numerous models have been formulated (1,17).

Lawson et al. (14) first demonstrated significant PVY resistance in potato. Two lines that had the potential to synthesize PVY CP were identified and displayed a high level of resistance against PVY, yet little of the expected transgene PVY CP was observed. Other lines that expressed higher and readily detectable

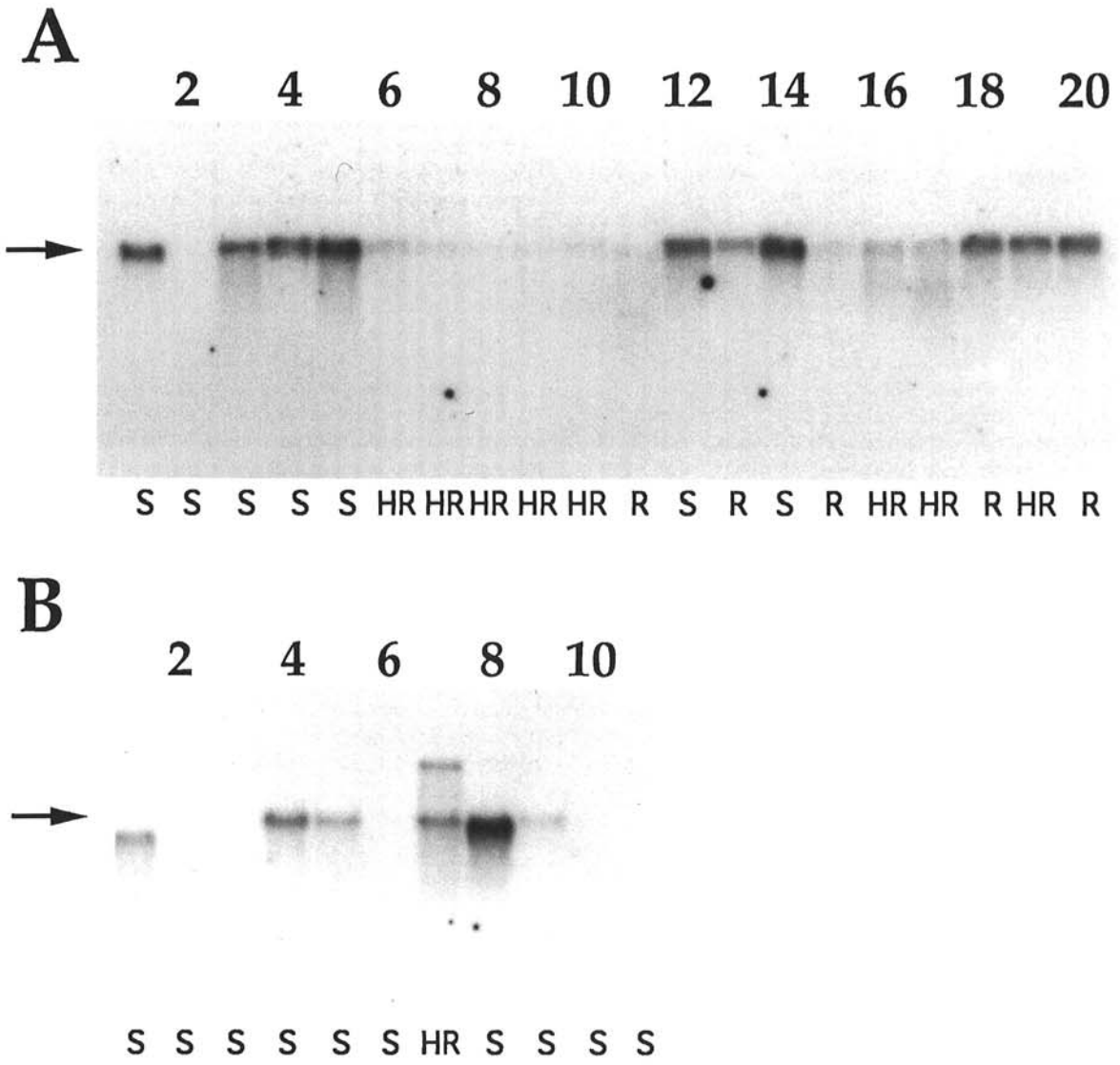

Fig. 2. Northern gel blot analysis of transcripts derived from transgenes containing potato virus Y (PVY) coat protein (CP) sequences. Total RNA was separated by electrophoresis in 1.2% agarose gels containing formaldehyde. The RNA was transferred to nitrocellulose and hybridized with a ^{32}P-labeled riboprobe complementary to **A,** sense or **B,** antisense (AS) PVY sequences. The arrow at the left indicates the position to which a ~1,100-nt transcript migrated. The phenotype of these plants when inoculated with PVY is presented below the lane as susceptible (S), resistant (R), or highly resistant (HR). **A,** An autoradiogram showing RNA that hybridized with a ^{32}P-labeled riboprobe complementary to the sense strand of the PVY CP open reading frame (ORF). Lane 1, RNA from uninfected 'Russet Burbank' to which 0.1 ng of a T7 polymerase-generated PVY CP sense transcript was added; lane 2, RNA from uninfected Russet Burbank leaf tissue; lanes 3–20, RNA from transgenic lines NCP 2-018, NCP 3-126, RCP 2-011, NCP 2-020, NCP 3-155, NCP 3-156, RCP 6-510, RCP 6-428, RCP 6-353, N4 3-212, N4 3-191, R4 2-035, N4 3-192, N4 3-210, N4 3-214, R4 0-009, R4 1-001, and R4 1-003, respectively. **B,** An autoradiogram showing RNA that hybridized with a ^{32}P-labeled riboprobe complementary to the PVY CP AS strand. Lane 1, total RNA from transgenic line RS 0-011 to which 0.1 ng of a SP6 polymerase-generated transcript was added; lanes 2–11, total RNA from transgenic lines RS 0-011, NA 1-004, NA 2-010, NA 3-079, NA 3-097, RA 2-002, RA 2-006, RA 3-066, RA 3-077, and RS 0-011, respectively.

levels of PVY CP were susceptible. These authors suggested that factors other than PVY CP level might be involved in protection or that the subcellular localization of the transgene-derived PVY CP was crucial.

van der Vlugt et al. (27) subsequently showed that a high level of PVY resistance could be generated in tobacco using transgenes that were either capable of expressing mRNA and PVY CP or had the initiation codon removed, rendering it untranslatable or able to produce only a severely truncated PVY CP. This is one of a few studies in which a sufficient number of transgenic plants were examined and data presented to allow a critical assessment of the conclusions. Resistant lines had multiple transgenes and accumulated steady state levels of the transcript to surprisingly low levels. van der Vlugt et al. (27) suggested that resistance was an RNA-mediated event possibly occurring via one of two mechanisms. The transgene transcript could hybridize with the minus strand intermediate synthesized during PVY replication. Additional studies permit us to suggest, however, that it is unlikely an ~1,250-nt transgene transcript, likely complexed with proteins, would successfully hybridize with a 10,000-nt viral RNA (5; C. Brown and W. G. Dougherty, *unpublished data*). It also has been found that lines accumulating this transgene RNA at a low level can show resistance, whereas lines accumulating this RNA at a higher level are susceptible. Both observations would argue against this hypothesis.

Another possible mode of action suggested by van der Vlugt et al. (27) is that transgene-derived RNA could titrate essential host or viral factors, making them unavailable for the virus replication process. Once again, with additional data (here and 25), it is difficult to reconcile the negative correlation between transgene RNA steady state levels and resistance with this possible mode of action.

Kollar et al. (12) reported that tobacco plants expressing the CP ORF derived from a Hungarian isolate of PVY displayed some resistance. No obvious relationship between CP steady state levels and resistance was noted, and the limited number of transgenes and plants examined preclude the formation of a hypothesis focusing on mode of action.

Farinelli and Malnoë (7) and Farinelli et al. (8), expressing a PVY nuclear inclusion b (NIb)-CP fusion, also were able to generate a limited number of lines that displayed a high level of resistance to PVY. A stop codon was introduced into this transgene construct such that a truncated protein comprised of the C-terminal 41 or 82 amino acids of NIb protein, 5 N-terminal amino acids of the PVY CP, and 14 amino acids not coded for by the PVY CP ORF could be made. A limited number of lines expressing this altered PVY sequence were resistant to PVY. Farinelli et al. (8) speculated that this altered transgene RNA and truncated product might function in one of three ways. First, the altered transgene RNA could hybridize with the PVY minus strand made during replication and arrest replication. As discussed above, this is difficult to envision, and the data available would not appear to support it. In a second scenario, the truncated protein product (not detected) could provide a cleavage site and titrate a PVY proteinase activity required for gene expression. Although possible, given that the nuclear inclusion a proteinase is synthesized in excess and is a processive enzyme (6), it may be difficult to effectively compete with this activity. Nevertheless, Farinelli et al. (8) propose a readily testable hypothesis. Finally, they suggest that CP might actually be synthesized by ribosomal frameshifting and that low levels of PVY CP function by interfering with the PVY replication cycle. As discussed above, it is difficult to reconcile this explanation with results obtained from plants that express readily detectable levels of the PVY CP yet are susceptible.

Recently, a collection of transgenic tobacco plants containing PVY transgenes expressing translatable and untranslatable mRNAs were examined (25). Highly resistant PVY tobacco plants had the hallmarks of RNA-mediated resistance detailed for transgenic TEV resistance (5,18). Resistant plants had multiple transgenes that were transcribed at a high rate, but accumulated the transcript at low steady state levels. It has been suggested that a host cell response, normally involved in the elimination of aberrant or overexpressed RNAs, has been programmed and is responsible for the resistance phenotype in these plants (5). The transgene serves to preactivate this sequence-specific RNA degradation pathway, and any RNA sharing this sequence (i.e., viral genome) also will be eliminated. The direct involvement of the transgene product(s) with the virus is unlikely.

The data in this manuscript are consistent with the highly resistant state reflecting a host cell response. With two exceptions, highly resistant lines expressing a sense transcript had multiple transgenes that accumulated the transgene product (RNA and/or protein) to low levels. Conversely, susceptible lines accumulated relatively higher levels of transgene transcript and CP. Some differences between our transgenic tobacco (25) and potato studies were noted. The transgenic PVY-resistant potatoes generated consistently had a higher number of transgene copies than did our transgenic tobacco, although the same binary vectors and *Agrobacterium* isolates were used for both. We also generated potato plants that permitted virus accumulation only in the inoculated leaves, not in distal leaves. This type of resistance was not noted in our PVY/tobacco study but may be an extreme type of the recovery phenotype observed with TEV in transgenic tobacco (18).

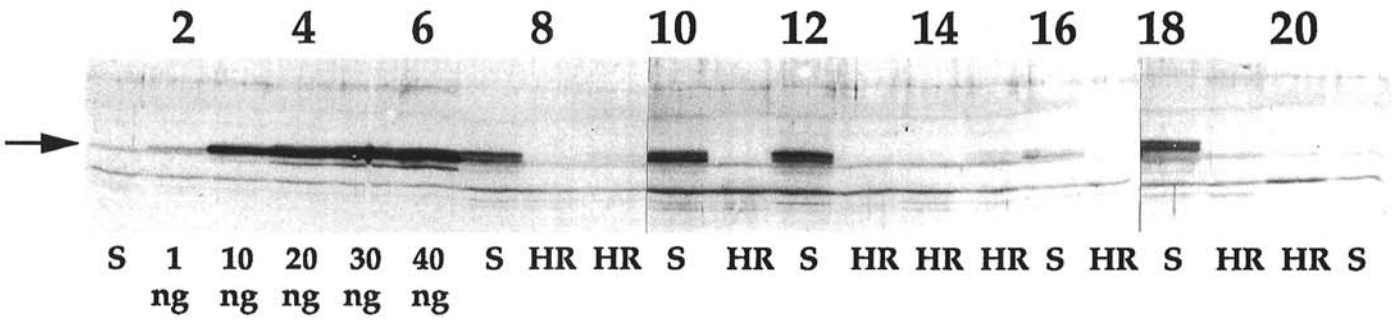

Fig. 3. Western gel blot analysis of transgenic potato leaf tissue expressing potato virus Y (PVY) coat protein (CP). Leaf tissue (2 mg) was ground in Laemmli dissociation buffer, and proteins in the clarified extracts were separated by electrophoresis in 10% polyacrylamide gels containing sodium dodecyl sulfate. Proteins were electroblotted to nitrocellulose membranes and reacted with polyclonal rabbit antiserum to PVY CP. The PVY CP antibodies were detected using an anti-rabbit antibody conjugated to alkaline phosphatase. The arrow at the left indicates the position of the PVY CP. Only a portion of the Western gel blot is shown. Lanes 1–6 represent transgenic line NS 2-053 to which 0.0, 1.0, 10.0, 20.0, 30.0, or 40.0 ng of PVY CP was added, respectively. Samples from other transgenic lines are as follows: lanes 7–21, NCP 2-018, NCP 2-019, NCP 2-020, NCP 3-128, NCP 3-132, NCP 3-134, NCP 3-136, NCP 3-138, NCP 3-144, NCP 3-152, NCP 3-155, RCP 6-432, RCP 6-510, NCP 3-156, and NS 2-053, respectively. The phenotype (susceptible [S], highly resistant [HR]) after inoculation with PVY is presented below lanes 1 and lanes 7–21. The amount (in nanograms) of PVY CP added in the reconstitution studies (lanes 2–6) is presented below those lines.

Alternatively, this type of response may be due to the additive effects of the transgenic resistance and a naturally occurring tolerance in these potato lines.

The observation of a high level of resistance associated with the RA 2-002 line was surprising. With one exception in our previous studies (15), plants containing AS transgenes have been susceptible. It is tempting to speculate that the higher molecular-weight transcript detected in these lines is responsible for the resistance phenotype. Future studies will focus on characterizing this RNA and attempting to segregate the sequence coding for this RNA away from the transgene coding for the expected AS RNA.

In summary, we have successfully transformed two cultivars of potatoes and have identified plants with the desired PVY-resistant phenotype. The highly resistant transformants have multiple transgenes and low steady state levels of the transgene transcript. The low steady state levels of the transgene product suggest the highly resistant state is not a dominant negative mutation phenotype, as originally proposed in pathogen-derived resistance, but rather is consistent with a host cell-mediated response.

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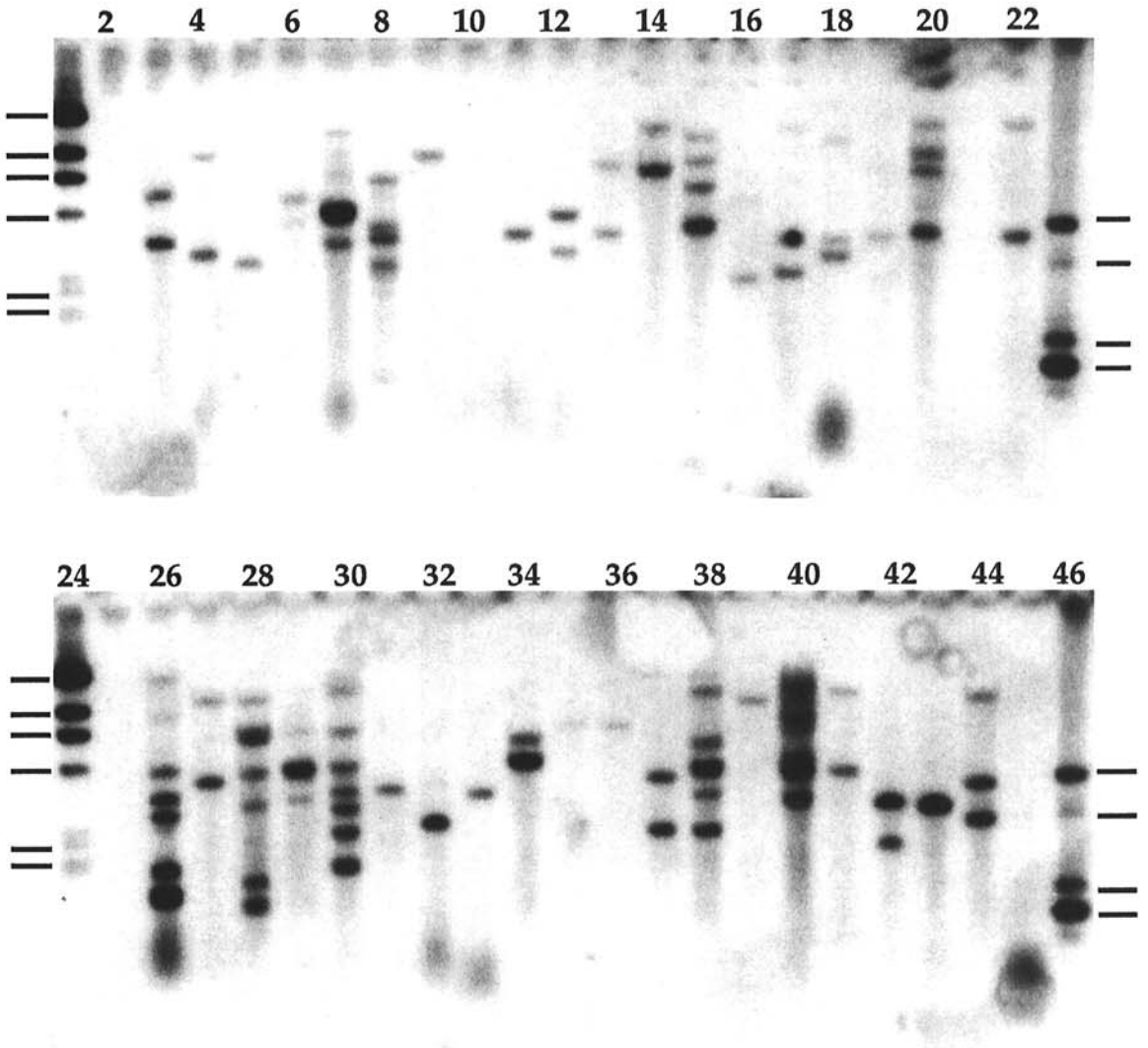

Fig. 4. Autoradiogram of Southern gel blot analysis of DNA from transgenic potato leaf tissue expressing various potato virus Y (PVY) transgenes. Genomic DNA was isolated and digested with the restriction endonuclease *Eco*RI. The resulting digestion fragments were separated by electrophoresis in 0.8% agarose gels, were blotted to nylon, and the filter was hybridized with ^{32}P-labeled DNA fragments specific for the PVY coat protein (CP) open reading frame (ORF) or λ-DNA. The DNA fragments were isolated from the following: lane 1, λ-DNA digested with *Hin*dIII: size of the DNA fragments in descending order is 23, 9.4, 6.6, 4.4, 2.3, and 2.0 kbp; lane 2, *Eco*RI-digested DNA from 'Russet Burbank'; lanes 3–22, transgenic lines R4 0-006, R4 1-002, R4 3-162, R4 5-389, RA 2-005, RA 3-066, RCP 2-011, RS 0-001, N1 2-033, N4 2-044, N4 3-164, N4 3-192, N4 3-210, NA 1-004, NA 3-079, NCP 2-019, NCP 3-128, NCP 3-136, NCP 3-139, and NCP 3-155, respectively; lane 23, Russet Burbank DNA to which 50 pg of PVY CP-related riboprobes of the following sizes were added: 4.3, 2.7, 1.3, and 0.9 kb; lane 24, same as lane 1; lane 25, Russet Burbank genomic DNA; lanes 26–45, transgenic lines R4 0-007, R4 2-035, R4 3-174, RA 2-002, RA 2-006, RA 4-264, RCP 6-432, N1 2-022, N4 2-043, N4 2-045, N4 3-191, N4 3-206, N4 3-214, NA 2-010, NA 3-097, NCP 3-126, NCP 3-134, NCP 3-138, NCP 3-144, and NS 2-053, respectively; lane 46, same as lane 23. Most of the DNA samples in lanes 21, 35, and 36 were lost in this particular experiment.

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