Coexpression of Potato PVY<sup>0</sup> Coat Protein and cryV-Bt Genes in Potato

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Abstract. The codon-modified cryV-Bt gene (cryV-Bt) from Bacillus thuringiensis subsp. kurstaki Berliner, which is specifically toxic to Lepidoptera and Coleoptera insects, and a potato virus Y<sup>0</sup> coat protein gene (PVY<sup>0</sup>cp), in which the aphid transmission site was inactivated, were cotransformed into potato (Solanum tuberosum L.) ‘Spunta’ via Agrobacterium tumefaciens Conn. We demonstrated the integration and expression of both genes by molecular analysis and bioassays. All cryV-Bt/PVY<sup>0</sup>cp-transgenic lines were more resistant to potato tuber moth (Phthorimaea operculella Zeller) and PVY<sup>0</sup> infection than nontransgenic ‘Spunta’. Four cryV-Bt/PVY<sup>0</sup>cp transgenic lines were equal in potato tuber moth mortality to a cryV-Bt transgenic line, but of these four only two lines were equivalent in PVY<sup>0</sup> titer levels to a PVY<sup>0</sup>cp-transgenic line. We identified two transgenic lines, 6a-3 and 6a-5, which showed greater resistance to potato tuber moth and PVY<sup>0</sup> than the other cryV-Bt/PVY<sup>0</sup>cp transgenic lines. This study indicated that multiple genes, conferring insect pest resistance and virus resistance, could be engineered into and expressed simultaneously in a potato cultivar.

Potato tuber moth (Phthorimaea operculella) and potato virus Y<sup>0</sup> (PVY), a member of the Potyviridae family, both cause severe damage to the cultivated potato (Solanum tuberosum). Yield losses of 30% to 70% as well as damage and rotting in storage can result from tuber moth infestation and a yield depression of up to 80% can result from PVY depending on the viral strain and the potato variety (Raman and Palacios, 1982). In addition to the application of agricultural chemicals, genetic changes in the potato plant by traditional breeding have been used for many years to reduce the damage of potato tuber moth and PVY (Ross, 1986). However, a limited source of genes conferring resistance to diseases and insects exists in potato germplasm. Due to the tetraploid (2n = 4x = 48) nature of potato, improvement by breeding is inefficient compared to that of diploid species. Thus, the introgression of natural resistance genes from potato germplasm for combined control of PVY disease and potato tuber moth has been a challenge for potato breeders.

Genetic engineering now provides an alternative tool to control PVY and potato tuber moth via insertion of resistance genes into agronomically useful cultivars. This approach has the advantage to preserve the desirable traits of the recipient cultivar, which could otherwise be lost through breeding and selection (Lawson et al., 1990). The cry1 and cryV insecticidal crystal protein genes of Bacillus thuringiensis subsp. kurstaki Berliner (Bt) bacteria have been cloned, sequenced, modified, and transformed into potato cultivars. These transgenic cultivars expressed a high level of resistance to potato tuber moth (Van Rie et al., 1994; Douches et al., 1998). Since the initial demonstration of coat-protein (cp) mediated resistance against tobacco mosaic virus infection in transgenic tobacco (Powell-Albel et al., 1986), several PVY<sup>0</sup>cp genes have been isolated from viruses and introduced into potato cultivars through Agrobacterium tumefaciens Conn-mediated transfer. The expression of viral coat protein genes in transgenic potato is very effective in protecting agronomically important potatoes against PVY (Lawson et al., 1990; Malnoe et al., 1994). Cotransformation of pest resistance and virus resistance has been demonstrated in tobacco (Liang et al., 1994) with promising results.

In an attempt to provide resistance to potato tuber moth and PVY in a major potato cultivar through genetic engineering, a codon-modified cryV-Bt gene [now designated cry1Ia1 under revised nomenclature (Crickmore et al., 1995)] and a PVY<sup>0</sup>cp gene were cloned into the same vector. This construct formed two independent gene expression cassettes, each regulated by their own CaMV 35S promoter (Fig. 1). The purpose of this research was to determine whether a multiple gene construct would effectively express both genes by conferring resistance to potato tuber moth and PVY at a level equivalent to transgenic lines expressing these genes independently. We also report on the greenhouse and laboratory bioassays to evaluate PVY and potato tuber moth resistance, respectively.

Materials and Methods

Agrobacterium transformation vector

Agrobacterium tumefaciens strain LBA4404 (Ooms et al., 1982) was used in the transformation experiments. The PVY<sup>0</sup>cp gene, which is harbored in the pBI121 vector (Clontech, Palo Alto, Calif.) and modified to inactivate the aphid transmission site, was obtained from T. German, University of Wisconsin,
used for amplification of a 0.5 kb fragment from the PVY\textsuperscript{cp} gene were as follows: upstream 5'-CTC GGGAAGTAGT TT-3' and downstream 5'-GAA CAC AGA GAG GCA CAC CGA-3'.

**Southern analysis.** The cryV\textsuperscript{-Bt}/PVY\textsuperscript{cp}-transgenic plants, nontransgenic cultivars, a cryV\textsuperscript{-Bt} transgenic ‘Spunta’ (G3), and a PVY\textsuperscript{cp}-transgenic ‘Spunta’ pBI-PVY\textsuperscript{cp} (Y1) were evaluated by Southern analyses. Genomic DNA (20 µg) was digested with BamHI to excise the cryV\textsuperscript{-Bt} gene for hybridization to the cryV\textsuperscript{-Bt} gene, and BamHI and SacI were used to excise the 1.2 kb PVY\textsuperscript{cp} gene for hybridization with the PVY\textsuperscript{cp} gene. Genomic DNA (20µg) which was digested with BamHI and hybridized with the PVY\textsuperscript{cp} gene was used to analyze copy number. The fragments were electrophoretically separated through a 1% agarose gel and transferred onto a nylon membrane (Hybond N, Amersham, U.K.).

**Northern analysis.** The cryV\textsuperscript{-Bt}/PVY\textsuperscript{cp}-transgenic plants, nontransgenic cultivars, two cryV\textsuperscript{-Bt}-transgenic ‘Spunta’ lines (G2, G3), and a PVY\textsuperscript{cp}-transgenic ‘Atlantic’ pBI-PVY\textsuperscript{cp} (Y1) were evaluated by northern analysis. Total RNA from young leaf and tuber tissues from greenhouse-grown plants were isolated using Qiagen RNeasy Plant Total RNA Kit (Qiagen, Chatsworth, Calif.). RNA (20 µg) along with ethidium bromide was fractionated by formaldehyde gel electrophoresis through a 1.0% agarose gel in MOPS buffer (20 mm MOPS, 5 mm sodium acetate, 1 mm EDTA, pH 7.0). Equal loading was observed by examining the ethidium bromide stained gel with an ultraviolet light. The gel was then blotted onto a Hybond N nylon membrane. Prehybridization, hybridization, and detection conditions were as described above for DNA analysis, except the membrane was exposed to X-ray film (Hyperfilm, Amersham) for 15 to 30 min.

**Production of transgenic plants.** ‘Spunta’ potato plants were micropropagated in GA-7 Magenta vessels each containing 25 mL of modified MS basal medium (Douches et al., 1998). The plants were grown at 23 to 27 °C in a 16 h photoperiod under fluorescent lights (30 µmol·m\textsuperscript{-2}·s\textsuperscript{-1}) for 2 weeks. The leaves, with tip and petiole ends removed, were then cultured abaxial side down on agar solidified step I medium (Yadav and Sticklen, 1995) for 2 to 4 d. The precultured explants were immersed for 5 to 10 min at room temperature in an A. tumefaciens LBA4404 (pBI606a) used for amplification of a 0.5 kb fragment from the PVY\textsuperscript{cp} gene were as follows: upstream 5'-CTC GGGAAGTAGT TT-3' and downstream 5'-GAA CAC AGA GAG GCA CAC CGA-3'.

![Fig. 1. Schematic figure of the plant expression vectors pBI-PVY, pBIML5, and pBIML6a used to express the PVY\textsuperscript{cp} and cryV\textsuperscript{-Bt} gene proteins in transgenic potato plants.](image)

The plasmid pBI121 was modified to remove the β-glucuronidase (GUS) gene. A codon-modified (J. Tippett, Garst Seeds, Slater, Iowa, personal communication) version of the cryV\textsuperscript{-Bt} gene (2.2 kb) (Tailor et al., 1992) was obtained from Garst Seeds/Zeneca (Berkshire, U.K.) and was inserted into the modified pBI121 plasmid to yield pBIML5. The pBIML5 vector was further modified by digesting with EcoRI (a site downstream of the NOS terminator) and blunt-ending by Klenow treatment. The linearized expression cassette of PVY\textsuperscript{cp} gene including a CaMV 35S promoter digested from pBI606a, was cloned into pBIML5 to create the 13 kb vector PBI606a (Fig. 1). The plasmids pBIML6a, pBIML5, and pBI-PVY\textsuperscript{cp} were mobilized into A. tumefaciens LBA4404 from E. coli by triparental mating (Bevan et al., 1984).

**Detection of PVY\textsuperscript{cp} gene and cryV\textsuperscript{-Bt} gene integration and expression.** PCR amplification. Genomic DNA was extracted from leaf tissue according to Saghai-Marof et al. (1984). The primers used for amplification of a 0.6 kb fragment from the cryV\textsuperscript{-Bt} gene were as follows: upstream 5'-AGC GCA AGC CAG TCC CTT CCC GCT TCA G-3' and downstream 5'-GGA CCA TCG GCG GCA CCC TCA ACA T-3'. The primers were used for amplification of a 0.5 kb fragment from the PVY\textsuperscript{cp} gene were as follows: upstream 5'-CTC GGGAAGTAGT TT-3' and downstream 5'-GAA CAC AGA GAG GCA CAC CGA-3'.

**Northern analysis.** The cryV\textsuperscript{-Bt}/PVY\textsuperscript{cp}-transgenic plants, nontransgenic cultivars, a cryV\textsuperscript{-Bt} transgenic ‘Spunta’ (G3), and a PVY\textsuperscript{cp}-transgenic ‘Spunta’ pBI-PVY\textsuperscript{cp} (Y1) were evaluated by northern analysis. Total RNA from young leaf and tuber tissues from greenhouse-grown plants were isolated using Qiagen RNeasy Plant Total RNA Kit (Qiagen, Chatsworth, Calif.). RNA (20 µg) along with ethidium bromide was fractionated by formaldehyde gel electrophoresis through a 1.0% agarose gel in MOPS buffer (20 mm MOPS, 5 mm sodium acetate, 1 mm EDTA, pH 7.0). Equal loading was observed by examining the ethidium bromide stained gel with an ultraviolet light. The gel was then blotted onto a Hybond N nylon membrane. Prehybridization, hybridization, and detection conditions were as described above for DNA analysis, except the membrane was hybridized at 52 °C and the cryV\textsuperscript{-Bt} and the PVY\textsuperscript{cp} gene probes were synthesized by in vitro RNA transcription using an RNA labeling kit (MBI, Indianapolis, Ind.).
PVY ocp Seeds, Slater, Iowa) at 1:60,000 dilution. The purified

Potato tuber moth bioassay

X-ray film (Hyperfilm, Amersham). instructions (BMB, Indianapolis, Ind.) and the blot was exposed to

binding was detected with CSPD according to the manufacturer’s

washing, the blot was subsequently probed with an alkaline phos-

protein was used as a standard along with Protein Molecular Weight

Bedford, Mass.) membrane in transfer buffer (25 mM Tris, 192 mM

Coomassie Brilliant Blue R-250 solution to visually compare load-

45 v and run overnight. One of the gels was then stained with

soluble leaf extracts. For each sample, 150 mg of total protein was

protein assay (BioRad, Hercules, Calif.) was conducted on the

Membrane was cut and placed in a 25

the excised leaves were placed in water-filled vials that were sealed

with a sponge to maintain plant turgor. The petri dishes were covered

and placed in a 25 ± 2 °C room with 25 μmol·m⁻²·s⁻¹ light. Each dish

represented one replication and each line was replicated four times. Mortality of the potato tuber moth larvae was determined after 72 h. Most mortality occurred in the first instar (1 mm), and when larvae had died and desiccated, it was not always possible to find the remains; therefore, missing larvae were considered dead.

**PVY o greenhouse bioassay**

The cryV-Bt/PVY cp-transgenic plants, nontransgenic cultivars, and a PVY cp-transgenic ‘Spunta’ were transplanted to a greenhouse with a 18 to 25 °C temperature range and a 16-h photoperiod (30 μmol·m⁻²·s⁻¹). When plants were at the five leaf stage, two well-expanded, topmost leaves were dusted with carbonudum (400 mesh) and rubbed with 150 μL sap (extracted using a mechanical grinder) from 2 g of potato leaves infected with PVY o virus and diluted 10:1 v/v with distilled water. After rubbing, the inoculated leaves were immediately rinsed with water. As a high selection stress, virus replication and spread were enhanced in these plants by maintaining a higher temperature in the greenhouse (25 to 27 °C). Leaves were collected and analyzed by a double antibody sandwich enzyme-linked immunosorbant assay (DAS ELISA) using an anti-PVY antibody from Agdia Inc. (Elkhorn, Ind.), according to manufacturer’s instructions, for the extent of PVY virus infection 30 and 45 d postinoculation (DPI). Absorbance values were read on a Bio-Tek Instrument EL-307 at 405 nm. The transgenic lines were defined as susceptible to PVY if the ratio of the optical density (O.D.) absorbance (405 nm) mean of the transgenic line to the O.D. mean of healthy control was 4:1 or greater (McDonald et al., 1994).

**Results and Discussion**

**Transformation and identification of transgenic plants**

To introduce resistance to potato tuber moth and PVY, the cryV-Bt and PVY cp genes were transformed into potato, an economically important cultivar grown in North Africa. Shoots emerged from callus after 30 d culture on regeneration medium supplemented with 50 mg·L⁻¹ kanamycin and 200 mg·L⁻¹ Timentin. More than thirty kanamycin-resistant plants were obtained from each construct. The putative integration of the cryV-Bt and PVY cp genes in ‘Spunta’ lines was assayed initially by PCR (Fig. 2). Southern analysis was carried out on five PCR-positive cryV-Bt/PVY cp-transgenic lines (with pBIML6a T-DNA), one PCR-positive PVY cp-transgenic line (with pBI-PVY T-DNA), and one cryV-Bt transgenic line (with pBIML5 T-DNA) (Fig. 3). Southern analysis also determined that each cryV-Bt/PVY cp-transgenic line tested contained at least one or two copies of the PVY cp gene per tetraploid genome (Fig. 4). To determine copy number a PVY probe was hybridized to genomic DNA digested with BanHI, which digests upstream of the PVY gene.

**Expression of cryV-Bt and PVY cp genes in transgenic potato plants**

Transgenic plants containing pBIML6a (cryV-Bt/PVY cp), pBI-PVY (PVY cp), and pBIML5 (cryV-Bt) were analyzed on northern blots for cryV-Bt and PVY cp transcription. The expected size for the cryV-Bt and PVY cp mRNAs are 2.2 and 1.2 kb,
respectively. The cryV-Bt and PVY\*cp transcription levels in the transgenic potato plants, isolated from both leaf and tuber organs, are shown in Fig. 5 and Fig. 6, respectively. Both cryV-Bt and PVY\*cp mRNA accumulated in the leaves, but the levels of transcript in the tuber of cryV-Bt in the cryV-Bt/PVY\*cp transgenic lines were less than the cryV-Bt (no PVY\*cp gene) line (G3). Moreover, the PVY\*cp transcript in the tuber was more consistent and greater among the lines than the cryV-Bt transcripts across the cryV-Bt/PVY\*cp transgenic lines. One possibility for this observation is that some tuber-specific genes may interfere with the expression of the cryV-Bt gene in certain positions in the genome. Of these lines, 6a-1, which had two copies of the double gene construct, had a much higher PVY\*cp transcript level and a much lower cryV-Bt level. As expected in the transgenic lines with the pBIMAL5 construct (cryV-Bt), only mRNA for cryV-Bt was detected and in the transgenic lines using the PVY\*cp construct, only PVY\*cp mRNA was detected.

Western blot analysis was used to determine the cryV-Bt protein accumulation in transgenic plants. A total of five cryV-Bt/PVY\*cp transgenic lines and two cryV-Bt-transgenic lines (G2 and G3) contained detectable amounts of the 81.2kDa (Sekar et al., 1997) cryV-Bt protein (Fig. 7). A band of lower molecular weight also reacts with the cryV-Bt antibody. This lower band appears to be nonspecific binding because it is present in the nontransgenic controls. The matching Coomasie stained gel (data not shown) revealed that equal amounts of protein were loaded for all samples except for G2 (lane 8), which had 25% more protein. The level of cryV-Bt protein expression in the cryV-Bt/PVY\*cp transgenic lines varied. For example, the cryV-Bt-transgenic (G3) line contained significantly more cryV-Bt protein than the other cryV-Bt-transgenic (G2) as well as the five cryV-Bt/PVY\*cp transgenic lines. Line 6a-1 exhibited a lower protein level than the other four cryV-Bt/PVY\*cp transgenic lines. Northern analysis (Fig. 5) showed that line 6a-1 had less transcript levels than the other lines. One explanation for the low cryV-Bt transcript and protein levels is that the position of its transgene in the genome could be effecting it transcriptionally and translationally.

Insecticidal activity of cryV-Bt gene in transgenic plants

Seven transgenic lines (5 cryV-Bt/PVY\*cp, 1 cryV-Bt and 1 PVY\*cp) and ‘Spunta’ were screened for insecticidal activity using a leaf bioassay against neonate potato tuber moth larvae. All cryV-Bt transgenic lines (alone or in combination with PVY\*cp gene) were lethal to potato tuber moth larvae with 70% to 87% mortality (Table 1). Conversely, in the PVY\*cp-transgenic line and nontransgenic ‘Spunta’, mortality was 10% to 13%. In general, the larvae that fed on transgenic plants containing the cryV-Bt/PVY\*cp or cryV-Bt construct were severely restricted in growth, development, and leaf mining with up to 87% mortality during the first three days of feeding. This level of potato tuber moth mortality was similar to or higher than the levels reported by Douches et al. (1998) using the cryV-Bt/GUS fusion gene alone. Other reports showed that the highest mortality for PTM is >70% by using the cryIA(b) or cryIA(b6) (Van Rie J. et al., 1994). We also observed a range of potato tuber moth mortality among the cryV-Bt/PVY\*cp-transgenic lines (70% to 87%). Even though 100% mortality was not achieved in the 72-h assay, larval growth was restricted and all larvae were dead after five days of feeding (data not shown). Transgenic line 6a-1, which expressed the lowest level of cryV-Bt protein in the western analysis, also exhibited significantly lower mortality of potato tuber moth (Fig. 7, Table 1), suggesting that the concentration of toxic protein in transgenic tissue is, as expected, strongly correlated with insect lethality. cryV-Bt and PVY\*cp genes, in our research, were both independently regulated by the CaMV 35S promoter. Since the same promoter was used, homology exists between the two promoters and could therefore lead to cryV-Bt gene transcriptional silencing in some transgenic lines (Matzke and Matzke, 1995). This could be one reason for the range of PTM mortality among cryV-Bt/PVY\*cp transgenic plants (70% to 87%).

Susceptibility of transgenic potato plants to infection by PVY*

To evaluate PVY virus resistance, cryV-Bt/PVY\*cp-transgenic lines and PVY\*cp-transgenic lines (Y-1, Atl-5) were challenged by sap inoculation of the PVY\* strain and compared to nontransgenic ‘Spunta’ and ‘Atlantic’. To determine whether the PVY antibody would react with the transgenic coat protein, Spunta cryV-Bt/PVY\*cp-transgenic lines, which were not infected with the virus were compared to the nontransgenic ‘Spunta’ and ‘Atlantic’. The uninfected transgenic cryV-Bt/PVY\*cp-transgenic lines gave O.D. absorbance values equal to the uninfected nontransgenic lines (data not shown). As shown in Table 2, all transgenic lines tested, except line 6a-4, manifested moderate to high tolerance to PVY virus infection at 30 DPI, while the control plants were infected with PVY. The symptoms paralleled PVY titer levels (data not shown). A range of PVY tolerance was observed among the cryV-Bt/PVY\*cp-transgenic lines. Of the five transgenic potato plants, isolated from both leaf and tuber organs, are shown in Fig. 5 and Fig. 6, respectively. Both cryV-Bt and PVY\*cp mRNA accumulated in the leaves, but the levels of transcript in the tuber of cryV-Bt in the cryV-Bt/PVY\*cp transgenic lines were less than the cryV-Bt (no PVY\*cp gene) line (G3). Moreover, the PVY\*cp transcript in the tuber was more consistent and greater among the lines than the cryV-Bt transcripts across the cryV-Bt/PVY\*cp transgenic lines. One possibility for this observation is that some tuber-specific genes may interfere with the expression of the cryV-Bt gene in certain positions in the genome. Of these lines, 6a-1, which had two copies of the double gene construct, had a much higher PVY\*cp transcript level and a much lower cryV-Bt level. As expected in the transgenic lines with the pBIMAL5 construct (cryV-Bt), only mRNA for cryV-Bt was detected and in the transgenic lines using the PVY\*cp construct, only PVY\*cp mRNA was detected.

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Our Southern analysis (Fig. 4), only one the steady state of the transgene (Smith et al., 1994). According to be attractive targets of methylation, which could be related to similar or complementary viral RNA. We observed a strong visual very specific degradation of both the transgene mRNA and the associated with posttranscriptional gene silencing which involves Sijen et al. (1996) have shown that RNA-mediated resistance is there is evidence for an RNA-mediated response for resistance. PVY ocp response was as effective as the PVY tolerance observed with the two transgenic lines still had low PVY titer levels. This level of Fig. 6. Northern analysis for tuber tissue. (A) Total tuber RNA hybridized with cryV-Bt gene RNA probe. (B) Total RNA hybridized with PVY\textsuperscript{cp} gene RNA probe. Lane 1 = Spunta (nontransgenic plant); lanes 2 to 6 = 6a-1 to 6a-5; lane 7 = Y-1 (transgenic plant with PVY\textsuperscript{cp} gene only); lane 8 = G-3 (transgenic plant with cryV-Bt only).

cryV-Bt/PVY\textsuperscript{cp}-transgenic lines, 6a-3 and 6a-5 expressed the highest tolerance to PVY 30 DPI. After 45 d postinfection, these two transgenic lines still had low PVY titer levels. This level of response was as effective as the PVY tolerance observed with the PVY\textsuperscript{cp}-transgenic lines (Y-1 and Atl-5).

Lawson et al. (1990) achieved complete resistance to PVX and PVY in potato using viral coat protein genes. In comparison, our control of PVY was relatively incomplete. PVY titer levels increased as DPI increased (Table 2). This could be due to the PVY\textsuperscript{cp} gene itself. The PVY\textsuperscript{cp} gene we used in our research was different from those used by Lawson et al. (1990) and Smith et al. (1995). Our PVY\textsuperscript{cp} lines (Table 2) as well as other PVY\textsuperscript{cp} lines (unpublished observation) did not show a greater resistance to PVY infection compared to our best cryV-Bt/PVY\textsuperscript{cp} line. It may be that this PVY\textsuperscript{cp} gene is not as effective as the other previously cloned PVY\textsuperscript{cp} genes on cp translation level or on the extent of posttranscriptional gene silencing.

Initially, it was thought that it was the coat protein that conferred resistance to potato virus Y (Lawson et al., 1990). In another study, it was shown that untranslatable PVY coat protein constructs produced resistant lines as well. They found that the PVY\textsuperscript{cp} transformants, which were highly resistant, had multiple copies of the transgene and a low steady state level of transcripts. Multiple copies of the PVY\textsuperscript{cp} gene may also appear to be attractive targets of methylation, which could be related to the steady state of the transgene (Smith et al., 1994). According to our Southern analysis (Fig. 4), only one cryV-Bt/PVY\textsuperscript{cp} transgenic line, 6a-1 had more than one copy (located in different positions) of the PVY\textsuperscript{cp} gene. Smith et al. (1995) also showed that there is evidence for an RNA-mediated response for resistance. Sijen et al. (1996) have shown that RNA-mediated resistance is associated with posttranscriptional gene silencing which involves very specific degradation of both the transgene mRNA and the similar or complementary viral RNA. We observed a strong visual signal for RNA transcripts in cryV-Bt/ PVY\textsuperscript{cp} transgenic lines (Fig. 5), but the variation in resistance to PVY is significant among these transgenic plants according to our ELISA results (Table 2). Additionally, we observed moderate to high resistance levels with high transcription levels, however it was not a complete resistance. It is therefore likely that the coat protein plays a moderate role in the resistance levels in our study. The low number of copies, copies not closely linked and possibly poor coding sequence homology between our PVY\textsuperscript{cp} lines and the PVY viral RNA could account for our variable and incomplete resistance to PVY\textsuperscript{o} infection.

The results described above demonstrate that the cotransformation of cryV-Bt and PVY\textsuperscript{cp} genes in an economically important potato cultivar is effective against both potato tuber moth and PVY. We identified two cryV-Bt/PVY\textsuperscript{cp} transgenic lines, 6a-3 and 6a-5, which possess high tolerance to PVY infection as well as a strong resistance to potato tuber moth. The insect bioassay and virus inoculation assays were laboratory and greenhouse based, respectively. Beneficial factors present in the field such as predators and adverse environmental conditions for the insect and virus will contribute to their control but were not factors considered in the current lab and greenhouse experiments. Thus, a stringent field test of these transgenic plants with potato tuber moth and PVY infestation will be conducted during the upcoming years in subtropical regions, where tuber moth and PVY are major pest problems (i.e., Egypt), to fully test their efficacy.

Table 1. Feeding assay of transgenic lines with potato tuber moth.

| Construct         | Transgenic lines | Mortality ( % ) | Mining\textsuperscript{c} |
|-------------------|------------------|-----------------|-----------------------------|
| pBIML6a           | 6a-1             | 70 b            | N                           |
|                   | 6a-2             | 77 c            | N                           |
|                   | 6a-3             | 87 d            | N                           |
|                   | 6a-4             | 80 c            | N                           |
|                   | 6a-5             | 80 c            | N                           |
| pBIML5            | Y-1              | 83 cd           | N                           |
| pBI-PVY           | Y-1              | 10 a            | Y                           |
| Spunta\textsuperscript{a} |                   | 13 a            | Y                           |

\textsuperscript{a}Mortality was tested by Fisher’s protected LSD. Mortality means with the same letter are not significantly different (P < 0.05).

\textsuperscript{b}N = mining ceased during the 72-h feeding period; Y = mining continued for the duration of the 72-h feeding period.

\textsuperscript{c}Nontransgenic control.

Table 2. PVY\textsuperscript{o} suspect analysis of transgenic lines by DAS ELISA.

| Transgenic lines | PVY\textsuperscript{o} suspect of DAS ELISA\textsuperscript{d} |
|------------------|-------------------------------------------------------------|
|                  | 30               | 45               |
| 6a-1             | +++             | +++             |
| 6a-2             | ++              | +++             |
| 6a-3             | +               | +++             |
| 6a-4             | ++              | +++             |
| 6a-5             | +               | +++             |
| Y-1              | +               | +++             |
| Atl-5            | +               | +++             |
| Spunta\textsuperscript{a} | +++            | +++             |
| Atlantic\textsuperscript{a} | +++            | +++             |

\textsuperscript{a}+++ = susceptible (titer in inoculated plant: healthy plant > 4:1); ++ = tolerant (titer in inoculated plant: healthy plant 3 to 4:1); + = highly tolerant (titer in inoculated plant: healthy plant 12:1).

\textsuperscript{b}Nontransgenic plants.
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