Abstract

Is Resistance to PVY In NIb Transgenic Tobacco due to Gene Silencing?

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Background
Resistance to *Potato virus Y* (PVY) was conferred in transgenic tobacco expressing the intact and truncated PVY NIb (polymerase) gene, but was not observed when the sequences encoding the GDD domain were deleted from the transgene, suggesting that the resistance was protein rather than RNAmediated [Audy et al. (1994) MPMI 7: 15-22]. However, the mechanism of the resistance was not investigated further. Here, we investigated whether the resistance mechanism operated by inhibiting replication and/or movement, whether resistance was associated with RNA silencing, and whether resistance and transgene expression were identical in different generations.

Methods
Plants were assessed for resistance to PVY at two different growth stages. Protoplasts were prepared from transgenic tobacco plants to investigate the effects of the PVY transgene on PVY replication. Plants and protoplasts were inoculated with PVY and PVY RNA respectively. Nucleic acids were extracted and analyzed by northern blot hybridization to detect PVY sequences.

Results
Two different lines of tobacco plants transformed with a transgene encoding the NIb protein of PVY were found to be susceptible (S) or resistant (R) to infection by PVY, respectively. In S plants, PVY accumulated to levels similar to those found in non-transformed plants. In R plants, resistance in younger plants was not fully established and PVY could be detected in inoculated leaves, but not in upper leaves. However, in more developed plants no PVY could be detected by either immunoblotting or nucleic acid hybridization in either inoculated or upper leaves. PVY RNA did not accumulate in protoplasts from such R plants. Northern blot analysis showed the constitutive presence of siRNAs to NIb sequences in R, plants. The steady-state levels of NIb transgene mRNA in R plants were not the same in two diffent generations, although resistance to PVY was observed in both generations.

Conclusions
The resistance was found to operate via inhibition of viral replication. The constitutive presence of siRNAs to NIb sequences in R plants is consistent with a mechanism of resistance based on RNA silencing, even though the steady-state levels of NIb transgene mRNA were only slightly lower in R than in S plants in Generation 1.

Is Resistance to PVY in NIb-Transgenic Tobacco due to RNA Silencing?

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Introduction

Different lines of tobacco plants transformed with a transgene encoding the NIb protein of the ordinary strain of *Potato virus Y* (PVY-O) were found to be susceptible (S) or resistant (R) to infection by PVY-O. Transgenic lines expressing different truncated forms of the *NIb* gene failed to induce resistance to PVY-O [Audy et al. (1994) MPMI 7: 15-22]. This result, and the fact that co-inoculation of R plants with PVY-O and *Cucumber mosaic virus* failed to break the resistance to the former (unpublished) suggested that the resistance mechanism was protein-mediated [Audy et al. (1994) MPMI 7: 15-22]. In light of subsequent work by others on RNA silencing in other systems, the mechanism of resistance to PVY in *NIb* transgenic R plants was re-examined.

Methods

Two consecutive generations of 20 to 25 day-old NIb transgenic R and S plants were inoculated with PVY-infected sap in detached leaf assays, and viral accumulation in the inoculated leaves was assessed by Western blotting. Nucleic acids were extracted from the same plants to quantify steady-state levels of the NIb transgene mRNA and of siRNAs to NIb sequences at the time of inoculation with PVY. Protoplasts prepared from NIb transgenic tobacco plants were transfected with PVY RNA, to investigate the effects of the NIb transgene on PVY replication. The P1/HCPro of PVY-O was transiently expressed in R plants by agroinfiltration. All RNA analysis was made by Northern blotting, using specific RNA probes.

Results

Accumulation of PVY in inoculated leaves of R and S plants

At 7 days post-inoculation, PVY accumulated in inoculated leaves of S plants at levels comparable to those of non-transgenic plants, whereas PVY accumulation in inoculated leaves of R plants was either not detectable or very poor (Figure 1A).

Steady-state levels of NIb transgenic mRNA in R and S plants.

The steady-state levels of transgenic NIb transcripts were only slightly lower in R than in S plants, in Generation 1, but were much higher in S than in R plants in Generation 2 (Figure 1B).

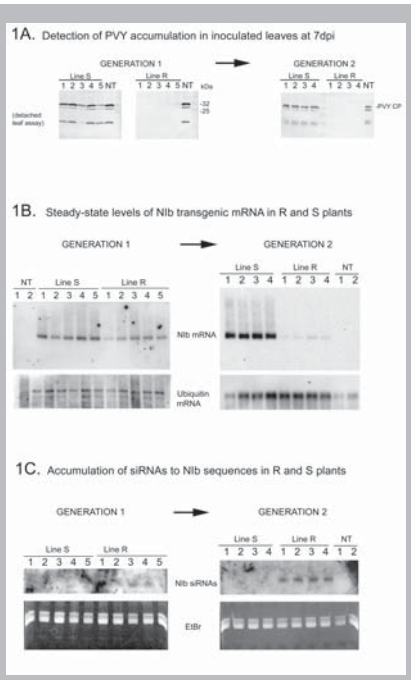

Figure 1A. Western blot analysis of PVY-O accumulation at 7 days post inoculation (dpi), in leaves of NIb transgenic susceptible (S), resistant (R), and non-transgenic (NT), plants. Two consecutive generations of plants were analysed. Each lane represents an individual plant. The Coat Protein (CP) of PVY was readily detected in NT and S plants, but barely or not at all detected in R plants.

Figure 1B. Northern blot analysis of the constitutive levels of NIb transgene mRNA in the plants analysed in 1A for susceptibility to PVY. Each lane represents RNA extracted from a single plant. NT = mRNA sample from non-transformed plant. Ubiquitin mRNA levels were assessed as a control for mRNA extraction (lower panel).

Figure 1C. Northern blot analysis of NIb siRNAs from same plants analysed in 1A and 1B. Each lane represents an individual plant. The lower panel is an ethidium bromide-stained gel shown as a control for loading. Note the constitutive presence of NIb siRNAs in extracts from R plants but not S plants (upper panel).

Accumulation of siRNAs to NIb sequences in R and S plants

The presence of siRNAs derived from a transgene is indicative of RNA silencing of the transgene. Little to no siRNAs to NIb sequences were detected in extracts from S plants in Generation 1 or 2, while such siRNAs were found in extracts from R plants of both Generations (Figure 1C).

PVY RNA accumulation in protoplasts from R and S plants

Resistance operating at the level of inhibition of virus replication was assessed by transfection of mesophyll protoplasts prepared from R and S transgenic plants. PVY RNA was able to accumulate in protoplasts made from S plants, but not from R plants (Figure 2, left). That R protoplasts were not refractory to transfection by viral RNA was shown by infection and accumulation of TMV RNA in R and S protoplasts (Figure 2, right).

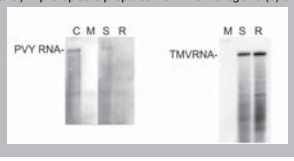

Figure 2. Northern blot analysis of RNA accumulation by PVY (left panel) and TMV (right panel) in protoplasts prepared from NIb transgenic (S) and (R) plants, electroporated with purified PVY RNA or TMV transcript RNA. M = mock inoculated non-transformed protoplasts, C = Total RNA sample from PVY-infected non-transformed tobacco. Note the absence of PVY accumulation in protoplasts prepared from R plants.

Effect of a silencing suppressor on NIb transgene mRNA accumulation in R and S plants

Transient expression by agroinfiltration of the PVY-O P1/HC-Pro silencing suppressor in leaves of Generation 2 R plants resulted in an increase in the level of the NIb mRNA (Figure 3).

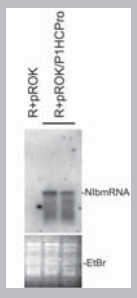

Figure 3. Northern blot hybridization analysis of NIb mRNAs from tissues expressing transiently the PVY suppressor of RNA silencing, P1/HC-Pro. Leaves of NIb R plants were agroinfiltrated with bacteria containing a plasmid harbouring either no insert (pROK) or the gene expressing the PVY P1/HC-Pro (upper panel). C = hybridization control of RNA extracted from a plant infected with PVY. The lower panel is an ethidium bromide-stained gel shown as control for loading. Note that P1/HC-Pro expression induced an increase in the steady-state level of the NIb transgene mRNA.

Conclusions

Detection of siRNAs derived from the NIb transgene mRNA in R, but not S plants suggests that RNA silencing of the transgene mRNA [and of any challenging homologous virus] is occurring in the R plants. That the NIb mRNAs constitutively transcribed in the R plants were being partially silenced was shown by the increase in its steady-state level following the transient expression of the PVY-O P1/HCPro suppressor of gene silencing. However, expression in *cis* of the same suppressor from inoculated PVY-O failed to counteract efficiently the resistance. Since the transgene sequences in both R and S plants are identical, it is difficult to imagine how a protein-mediated component of resistance can operate in the R, but no in the S plants. The data therefore indicate that RNA silencing is responsible for most and possibly all of the resistance to PVY in the R plants, and that the extent of RNA silencing of the transgene was greater in Generation 2 than in Generation 1.