Is Resistance to PVY In Nb-Transgenic Tobacco due to RNA Silencing?

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Abstract

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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-Nib) 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fact that co-inoculation of R plants with PVY-O and Cucumber mosaic virus failed to break the resistance to the former (unpublished) suggested that the resistance mechanism was protein-mediated [Audy et al.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 Nb transgenic R plants was re-examined.

Methods

Two consecutive generations of 20 to 25 day-old Nb 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 Nb transgene mRNA and of siRNAs to Nb sequences at the time of inoculation with PVY. Protoplasts prepared from Nb transgenic tobacco plants were transfected with PVY RNA, to investigate the effects of the Nb 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 Nb transgenic mRNA in R and S plants

The steady-state levels of transgenic Nb 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).

Accumulation of siRNAs to Nb sequences in R and S plants

The presence of siRNAs derived from the transgene is indicative of RNA silencing of the transgene. Little to no siRNAs to Nb 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).

Conclusions

Detection of siRNAs derived from the Nb 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 Nb 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 not 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.