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

Bong Nam Chung, Tomas Canto, and Peter Palukaitis Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK



## Summary

The mechanism of resistance to infection by *Potato virus* Y (PVY) in transgenic tobacco expressing the full-length PVY *Nlb* gene was analyzed. PVY RNA did not accumulate in protoplasts made from transgenic resistant (R) plants, but did accumulate in protoplasts made from transgenic susceptible (S) plants, indicating that resistance operated via inhibition of virus replication.

Examination of the steady-state transgene mRNA levels showed similar levels of NIb mRNA present in nucleic acids extracted from R and S plants. On the other hand, in non-inoculated plants, siRNAs to the transgene were observed only in nucleic acids extracted from R plants, while infection by PVY increased the level of siRNAs from PVY sequences in both R and S plants. Agroinfiltration with a plasmid expressing the silencing suppressor HC-Pro also led to an increase in the steady-state NIb mRNA level in R plants, but not in S plants.

Together, these data suggest that the mechanism of resistance is due in part to RNA silencing. However, it also was observed that while PVY accumulated to similar levels in S plants and non-transformed plants, the patterns of PVY accumulation in R plants, reflecting the extent of resistance, was age dependent: In younger plants (~25 days old), resistance was not fully established and virus accumulation could be detected in the inoculate leaves, but not in upper leaves, while in older plants (~40 days old), PVY accumulation could not be detected in the inoculated leaves by either immunological methods or nucleic acid hybridization.

Whether aging affects the steady-state levels of transgene mRNA and accumulation of siRNAs has not been established for this system. Therefore, at present, the data are not unequivocal as to whether the mechanisms of resistance is due to RNA silencing alone, or whether more than one mechanism is involved in the resistance, depending on plant age.

## **PVY** replication in R and S protoplasts

Resistance operating at the level of inhibition of virus replication was assessed by

transfection of mesophyll protoplasts prepared from the R and S transgenic plants [Figure 2]. PVY RNA was able to accumulate in protoplasts made from S plants, but not from R plants. That the R protoplasts were not refractory to transfection by viral RNA was shown by infection and accumulation of TMV RNA in R and S protoplasts. Therefore, the mechanism of resistance to PVY operates at the level of inhibition of virus reolication.



## Accumulation of NIb siRNA in R and S plants

The presence of siRNAs derived from a transgene is indicative of RNA silencing of the transgene. The presence of siRNAs in the R and S plants was assessed by northern blot

hybridization analysis. The NIb siRNAs were not detectable in RNAs extract from S plants, except after infection by PVY, while such siRNAs were detectable in extracts from R plants [Figure 4]. PVY infection did occur in these R plants and greatly increased the level of siRNAs, but not to the levels seen in PVY-infected S plants. Therefore, siRNAs were detected in R plants and not S plants prior to infection. This suggests that RNA silencing has reduced the level of NIb transgene mRNA in R plants vs. S plants.



### 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 [Figure 1]. Based on expression of

based on expression of different truncated forms of the *NIb* gene, the resistance was thought to be protein mediated [Audy et al. (1994) MPMI 7: 15-22]. However, the mechanism of resistance was not characterized further. In light of subsequent work by others on RNA silencing in other systems, the mechanism of resistance to expression of the full-length *NIb* transgene was re-evaluated here, using several approaches.



Figure 1. Infection of NIb-transpenic tobacco resistant (R) or susceptible (S) to PVY. Vein-clearing symptoms of infection b PVY are observed only in upper leaves of S transgenic plants, but not in upper leaves of R transgenic plants

#### Expression levels of NIb transgene mRNA in R and S plants

The steady-state levels of NIb transgene mRNA were assessed by northern blot hybridization analysis of mRNAs extracted from R and S plants. In mRNAs extracted from 25 day-old plants [Figure 3A], the steady-state levels of NIb transcripts were the same in R and S plants. This was also true in mRNAs extracted from 35 day-old R and S plants [Figure 3B]. These data suggest that resistance is not due to RNA silencing, unless the transgene mRNAs have not yet become targeted by RNA silencing.



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

If the NIb mRNAs in the R transgenic plants were being targeted for partial silencing, then the level of transgene expression would be expected to increase if a suppressor of RNA silencing was expressed in the same tissues.

Transient expression of the PVY P1/HC-Pro silencing suppressor in leaves of the R and S plants was achieved by agroinfiltration and the level of the NIb mRNA increased in R plants, but not in S plants [Figure 5]. This indicates that RNA silencing operates, at least in part, in this system, and that the level of expression of the transgene in the R lines is probably higher than in the S lines



Effect of plant age on resistance to PVY

The inability of PVY RNA to replicate in protoplasts prepared from fully expanded leaves of from R plants [Figure 2], but to accumulate in the inoculated leaves of R plants [Figure 4] is in contradiction. To examine whether the age of the plant affected the level of resistance, the ability of PVY to accumulate in the inoculated leaves of R and S plants at two different ages was assessed by western blotting [Table 1]. In younger R plants, PVY accumulate in the inoculated leaves, although to a lower level than in S plants or non-transformed plants, while in older R plants, PVY did not accumulate to detectable levels. Therefore, the extent of resistance to PVY infection depended on plant age.



## Conclusions

Resistance to PVY in transgenic tobacco expressing the PVY NIb gene operates at the level of inhibition of virus replication in older plants. In younger plants, resistance prevents systemic infection but allows virus accumulation in the inoculated leaves. Detection of siRNAs derived from the NIb transgene mRNA in the R plants but not the S plants indicates that RNA silencing of the transgene mRNA is occurring in the R plants. This is supported by an increase in the steady-state mRNA levels in R plants after expression of a suppressor of RNA silencing. Whether aging affects the steady-state levels of transgene mRNA and accumulation of siRNAs has not been established. Therefore, at present, the data are not unequivocal as to whether the mechanisms of resistance is conferred by RNA silencing alone, or whether more than one mechanism is involved in the resistance, depending on plant age.