Multiple pathways of resistance in tobacco to Tobacco mosaic virus

Peter Palukaitis, Minoru Takeshita, Ju-Yeon Yoon and Tomas Canto
Scottish Crop Research Institute, Dundee, DD2 5DA, UK

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Scottish Crop Research Institute

Summary

Resistance in tobacco to Tobacco mosaic virus (TMV), mediated through the *N* gene, involves a hypersensitive response (HR), as well as an inhibition response preventing further spread from the infected cells. The inhibition response was shown to involve at least three pathways, two [inducing an RNA-dependent RNA polymerase (RdRp-1) and an inhibitor of virus replication (IVR)] activated by salicylic acid (SA) and one SA-independent response mediated by the protein ERF5. TMV used as a vector for gene expression is more sensitive to the ERF5 response than wildtype TMV; ERF5 affects both the local and systemic movement of TMV vectors. The ERF5 response could be neutralized by the Cucumber mosaic virus (CMV) 1a protein, which binds to ERF5. The RdRp-1 and IVR responses could individually be neutralized by transgene-mediated RNA silencing. Both the RdRp-1 and IVR responses are involved in systemic acquired resistance (SAR) activated by SA, as shown by RNA silencing. Therefore, *N* gene-mediated resistance induces multiple pathways, each contributing to partial inhibition of the virus.

Background

The *N* gene-mediated resistance response in tobacco to infection by TMV restricts the virus to a necrotic lesion produced by the HR. RdRp-1 [1] and IVR [2] may be involved in this resistance, since expression of either reduces TMV accumulation. TMV vectors are restricted further and the lesions are not visible. TMV vectors also are blocked from systemic infection of *N* gene tobacco above 28 °C, when the *N* gene-mediated resistance is not active against TMV. Transgenic expression of the CMV 1a protein neutralizes both of these novel resistance responses, but does not prevent the HR [3]. Moreover, transgenic tobacco plants in which the SA-mediated defense response is compromised by expression of the SA-destroying enzyme, salicylate hydroxylase (nahG plants), do not show visible, spreading lesions after infection by TMV vectors, unless the CMV 1a protein also is present [3]. This demonstrates that the CMV 1a does not neutralize the SA-mediated defense response, but rather a novel defense response. This work examines factors involved in the SA-mediated and SA-independent defense responses to infection by TMV and TMV vectors.

Silencing the genes for RdRp-1 or IVR reduces resistance to TMV in tobacco

Transgenic tobacco (*Nicotiana tabacum* cv. Samsun NN) containing the *N* gene were prepared to express inverted-repeat hairpin (ih) constructs of part of either the RdRp-1 gene [1] or the IVR gene [2]. These plants were silenced for the expression of the corresponding plant genes (not shown). Infection of either type of transgenic plant led to local infection followed by limited systemic movement of TMV at ambient temperature [Figure 1], indicating that part, but not all, of the inhibition response had been compromised.

Fig. 1. Infection of tobacco with TMV. In non transformed plants (left) infection (necrotic lesions) is limited to the inoculated leaves. In plants compromised for expression of RdRp-1 (middle) or IVR (right), necrosis is seen in the stem (middle plant) and upper leaves (middle and right), showing a partial loss of resistance.

The ih-IVR or the ih-RdRp-1 transgenic plants inoculated with TMV, two days after treatment with SA, were more susceptible to infection than non transformed plants [Figure 2]. This shows that the SAR also was compromised in part by silencing of either IVR or RdRp-1.

Fig. 2. Inhibition of systemic acquired resistance (SAR). Transgenic plants compromised for expression of either RdRp-1 or IVR were watered with a solution of SA for two days prior to inoculation with TMV. Those plants not transformed produced few and smaller lesions than the plants in which expression of either RdRp-1 or IVR was inhibited, indicating inhibition of the SAR response.

The CMV 1a protein interacts with ERF5 and inhibits resistance to TMV

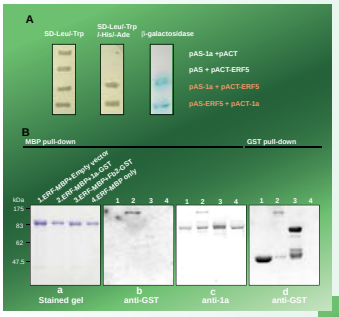

Fig. 3. Interaction between the CMV 1a protein and *Nt*ERF5. (A) A yeast two-hybrid screen identified the *Nt*ERF5 as interacting with the CMV 1a protein. (B) Pull-down assay of MBP-tagged *Nt*ERF5 and GST-tagged CMV 1a protein by immunoprecipitation with anti-MBP antibody (a) and detection with either anti-GST (b) or anti-1a antibody (c). Controls: pull-down of the same complexes with anti-GST and detection with anti-GST (d); FB_1-GST = Fibrillarin, a nuclear protein.

The yeast two hybrid assay was used to screen a *Nicotiana tabacum* (NN geneotype) cDNA library for proteins interacting with the CMV 1a protein. One such interacting tobacco protein was ethylene response factor 5 (ERF5) [Figure 3A] [4]. The CMV 1a protein also interacted with ERF5 *in vitro* [Figure 3B].

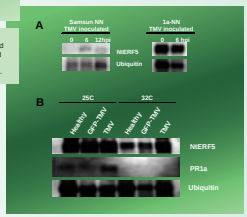

Fig. 4. Gene expression of *Nt*ERF5. (A) Non transformed (Samsun NN) or CMV 1a-transgenic tobacco (1a-NN) inoculated with TMV showed induction of ERF5 in the non transformed plants, but constitutive expression of ERF5 in the 1a-NN plants. (B) CMV 1a transgenic plants, not inoculated (Healthy), or infected with TMV or GFP-TMV (a TMV vector expressing the green fluorescent protein) and incubated at either 25 °C or 32 °C all showed expression of ERF5.

ERF5 is known to be involved in the defense response to infection by TMV and is induced by infection with TMV [4; Figure 4A], but not by ethylene [4]. Surprisingly, the CMV 1a protein also stimulated the expression of ERF5 [Figure 4].

CMV 1a plants crossed with nahG plants (with compromised SA-mediated resistance) gave progeny plants that showed less resistance to TMV than nahG plants [Figure 5].

Fig. 5. Enhanced susceptibility to TMV infection. Transgenic nahG tobacco compromised for SA-mediated defense (right) was more resistant to infection by TMV than a nahG tobacco also expressing the CMV 1a protein (left).

Conclusions

Inhibition of systemic movement of TMV could be compromised in part by silencing gene expression of IVR or RdRp-1. This suggests that together these proteins engender the strong resistance associated with expression of the *N* gene in tobacco. This probably functions through SAR, since these proteins also participate in the SA-induced SAR response. ERF5 is also involved in this resistance response, but to a lesser extent [4]. Since the CMV 1a protein in transgenic tobacco plants stimulated both ERF5 expression and the movement of TMV vectors [3], we conclude that the CMV 1a protein must be neutralizing ERF5, which is believed to be a transcription factor involved in SA-independent resistance to TMV [4].

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