Research Note

Tobamovirus Coat Proteins: Elicitors of the Hypersensitive Response in *Solanum melongena* (Eggplant)

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***Solanum melongena* (eggplant) exhibits a hypersensitive response (HR) when infected with tobacco mosaic tobamovirus (TMV). In contrast, a TMV mutant unable to express coat protein (CP) did not elicit the HR, while a potexvirus vector engineered to express TMV CP did elicit the eggplant HR. The CPs of U2 and odontoglossum ringspot tobamoviruses also elicited the HR. However, the HR was not elicited by the CP of cucumber green mottle mosaic tobamovirus. Taken together, these findings demonstrate that specific tobamovirus CPs function as elicitors of the eggplant HR.**

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Plants maintain sophisticated surveillance mechanisms that recognize and respond to specific pathogens. Recognition often leads to the induction of a cascade of host defense responses that result in localized cell death and disease resistance. This form of resistance, termed the hypersensitive response (HR), typically involves the interaction of specific pathogen avirulence genes (*avr*) with specific plant resistance genes (*R*). Models for this gene-for-gene interaction predict that plant *R* genes encode receptors that bind elicitors encoded by pathogen *avr* genes (Lamb et al. 1989; Gabriel and Rolfe 1990; Keen 1992). Molecular characterizations of both pathogen elicitors and plant *R* genes have revealed a number of features suggestive of such a receptor-ligand interaction (Staskawicz et al. 1995; Bent 1996). In addition, direct evidence for a receptor-elicitor interaction has been demonstrated in at least one system (Scofield et al. 1996; Tang et al. 1996).

For tobamoviruses, several dominant or partially dominant plant *R* genes conferring similar HRs have been genetically identified (Holmes 1938, 1946) and one *R* gene, *N* from *Nicotiana glutinosa*, has been molecularly cloned (Whitham et al. 1994). The viral determinants involved in eliciting some of these HRs have also been identified. They include the coat protein (CP), which elicits HRs conferred by the *N'* gene in *N. sylvestris* and the *L*³ or *L*² genes in pepper (Culver and Dawson 1991; Berzal-Herranz et al. 1995; de la Cruz et al. 1997);

the 30-kDa cell-to-cell movement protein, which elicits HRs conferred by the *Tm-2* and *Tm-2*² genes in tomato (Meshi et al. 1989; Weber et al. 1993); and the 126-kDa replicase protein, which elicits the HR conferred by the *N* gene in *N. glutinosa* (Padgett and Beachy 1993). Host range studies also indicated the presence of additional tobamovirus-elicited HRs in other plant hosts (Holmes 1946). Thus, tobamoviruses provide an ideal system for comparing the mechanisms used by different hosts in pathogen recognition.

In this study, the ability of tobacco mosaic tobamovirus (TMV) to elicit necrotic local lesions in *Solanum melongena* (eggplant) was investigated (Holmes 1946). Eggplant leaves inoculated with TMV developed necrotic lesions at the sites of viral invasion within 2 to 3 days postinoculation (Fig. 1C). These lesions expanded slowly over time and were followed by abscission of the inoculated leaves within 2 to 3 weeks postinoculation. To determine whether the TMV CP was involved in eliciting this response, infectious RNA derived from a full-length TMV cDNA construct missing the CP translational start codon (pTMVNS) (Culver 1996) was used to mechanically inoculate leaves of the eggplant cultivar Black Beauty (Ferry-Morse Seed Co., Fulton, KY). TMVNS has been shown to replicate and move from cell to cell at levels and rates comparable to those of wild-type TMV (Culver 1996; Culver and Dawson 1989). All plants were maintained in environmental growth chambers at 25°C under a 12-h photoperiod and monitored for symptom development for a term of at least 3 weeks. To confirm the presence of TMVNS in infected tissues, Western immunoblots were conducted to detect the TMV 126-kDa replicase protein (Fig. 2). Infected leaf tissue was pulverized directly in sample loading buffer and boiled for 5 min (Laemmli 1970). Sample proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose paper. Transferred proteins were probed with either rabbit anti-126-kDa replicase antibodies or anti-CP antibodies (Lehto et al. 1990) followed by alkaline phosphatase-conjugated goat anti-rabbit antibody (Sigma, St. Louis, MO). Antibody-bound proteins were visualized by the addition of BCIP-NBT (5-bromo-4-chloro-3-indolyl-phosphate toluidinium-nitroblue tetrazolium) (Knecht and Dimond 1984). Results showed that TMVNS replicated in inoculated eggplant tissue but did not elicit the HR (Fig. 2). This

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Fig. 1. Symptomatology of tobamovirus coat proteins (CPs) in eggplant. **A**, Tobacco mosaic tobamovirus (TMV) construct TMVCP^{U2} at 3 days postinoculation (dpi); **B**, TMVCPO (TMV construct expressing odontoglossum ringspot tobamovirus CP) at 6 dpi; **C**, TMVCP^{CGMMV} (TMV construct expressing cucumber green mottle mosaic tobamovirus CP, right side) and wild-type TMV (left side) at 14 dpi; and **D**, potato X potexvirus (PVX) constructs PVX2C2S (right side) and PVXCP^{TMV} (PVX expressing TMV CP, left side) at 14 dpi.

finding demonstrates a requirement for CP expression in the TMV elicitation of the eggplant HR.

To determine whether TMV CP functioned alone or in conjunction with other TMV components to elicit the eggplant HR, a potato X potexvirus (PVX) vector, PVX2C2S (Chapman et al. 1992), was used to express the TMV CP. The TMV CP open reading frame was cloned into the unique *EcoRV* and *SalI* sites of pPVX2C2S to create pPVXCP^{TMV} (Culver 1996). Expression of the TMV CP in eggplant by PVXCP^{TMV} was confirmed via Western immunoblot analysis (Fig. 3, lane 6). Eggplant leaves inoculated with PVXCP^{TMV} developed necrotic lesions within 4 days, while leaves inoculated with the empty vector construct, PVX2C2S, displayed no visible symptoms (Fig. 1D). The local lesions elicited by PVXCP^{TMV} generally appeared more slowly and were less defined than lesions elicited by TMV. This may be the result of differences in the levels and timing of CP expression between TMV and the PVX vector. The ability of PVXCP^{TMV} but not of PVX2C2S to elicit the HR demonstrates that TMV CP expressed from a heterologous virus is sufficient to elicit the eggplant HR.

The HRs conferred by the tobacco *N'* gene and the pepper *L*¹, *L*², and *L*³ genes are similarly elicited by CPs from a diverse group of tobamoviruses, including U2, odontoglossum ringspot (ORSV), and cucumber green mottle mosaic tobamoviruses (CGMMV) (Taraporewala and Culver 1997; C. D. Dardick, unpublished data). The ability of eggplant to recognize these different CPs was investigated by using TMV constructs containing substituted CP open reading frames from either U2 (TMVCP^{U2}), ORSV (TMVCPO), or CGMMV

Fig. 2. Western immunoblot detection of tobacco mosaic tobamovirus (TMV) 126-kDa protein and coat protein (CP) in tobacco tissue inoculated with TMV U1 (lanes 1 and 3) and eggplant tissue inoculated with TMVNS (lanes 2 and 4).

(TMVCP^{CGMMV}) (Hilf and Dawson 1993; Culver 1996; Taraporewala and Culver 1997). CP expression in eggplant was monitored through Western immunoblot analysis (Fig. 3) (CGMMV antisera from the ATCC, Rockville, MD). CPs from U2 and ORSV were found to elicit an HR similar to the one elicited by the wild-type U1 CP (Fig. 1A and 1B). In contrast, the CP of CGMMV did not elicit the HR but instead induced spreading chlorosis within the inoculated leaf (Fig. 1C). It is interesting to note that the CP of CGMMV elicits the *N'* gene HR in tobacco, while the U1 CP does not elicit this response (Taraporewala and Culver 1997). This variation in the abilities of different CPs to act as elicitors in different hosts suggests that these plants target different CP features for recognition.

Fig. 3. Western immunoblot detection of coat protein (CP) expression in inoculated eggplant tissue. Lane 1, healthy control; lane 2, tobacco mosaic tobamovirus (TMV) U1; lane 3, TMVCP^{U2} (TMV construct expressing U2 CP); lane 4, TMVCPO (TMV construct expressing onion-toglossum ringspot tobamovirus CP); lane 5, potato X potyvirus (PVX) construct PVX2C2S; lane 6, PVXCPTMV (PVX expressing TMV CP); and lane 7, TMVCP^{CGMMV} (TMV construct expressing cucumber green mottle mosaic tobamovirus CP).

The structural features of the tobamovirus CP responsible for eggplant recognition remain unknown. However, features along the right face of the CP helical bundle, comprising a hydrophobic cavity and surrounding charged residues, were shown to be critical in the elicitation of the *N'* gene HR in *N. sylvestris* (Taraporewala and Culver 1996). Further analysis of the structural and biophysical features of CP involved in eliciting the eggplant HR and other host *R* genes should result in a better understanding of the different mechanisms utilized by plants in pathogen recognition.

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