Evaluation of the coat protein of the *Tomusviridae* as a HR elicitor in *Nicotiana* section *Alatae*

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Plants are able to recognize and respond to virus infection with a hypersensitive response, a plant defense response that triggers a cell death pathway and limits the viral infection to the infected leaf. We have previously shown that the coat protein of *Tomato bushy stunt virus* (TBSV) and *Tobacco necrosis virus* strain DH (TNV-DH) trigger HR in several species of *Nicotiana*, including *Nicotiana langsdorffii*. To identify structural features in the coat protein recognized by the host, we tested the capacity of eight coat proteins of the *Tomusviridae* to trigger a HR in *N. langsdorffii*. These eight coat proteins represented six genera of the *Tomusviridae*. We found that the coat proteins of TBSV, *Cucumber Necrosis Virus*, *Cymbidium ringspot virus*, *Red Clover necrotic mosaic virus* and TNV-DH triggered HR, whereas the coat proteins of *Turnip crinkle virus*, *Maize chlorotic mottle virus* and *Panicum mosaic virus* did not. A comparison of the amino acid sequences of all eight coat proteins revealed that only two amino acids (Asp155 and Arg161) were conserved amongst the coat proteins that triggered HR and distinguished them from the coat proteins that did not trigger HR. Computer modeling of the coat protein structure allowed for placement of these two amino acids within the three dimensional structure of the coat protein and suggested how mutations might affect the overall structure. Site directed mutagenesis of these two amino acids in the TNV-DH coat protein abolished HR, demonstrating that they have an essential role in recognition and elicitation of plant defenses against this virus.