Public Abstract

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Title:CHARACTERIZATION OF RESISTANCE TO TOMBUSVIRUS IN *NICOTIANA* SPECIES

Resistance to plant viruses has been explained by the gene for gene model, which requires the recognition between a host resistance(R) gene and a viral avirulence (Avr) gene, resistance that is commonly associated with a hypersensitive response (HR). Our lab has been characterizing resistance to Tombusviruses in *Nicotiana* species following this model. On the host side, we previously showed that dominant resistance to several tombusviruses such as Tomato bushy stunt virus (TBSV), Cymbidium ringspot virus (CymRSV), and Cucumber necrosis virus (CNV) can be introgressed from *N. glutinosa* into the susceptible host *N. clevelandii* through the development of an addition line which we designated *N. clevelandii* Line 36. Recently, we showed that posttranscriptional silencing of the *N* gene not only affected the HR elicited by the tobamovirus Tobacco mosaic virus (TMV), but also the HR to TBSV and CymRSV. We hypothesized that the R gene against tombusviruses could be a member of the *N* family of R genes present in *N. glutinosa* and distinct from it. In this study, I characterized the family of *N* gene homologs (NGHs) in *N. glutinosa*, *N. clevelandii* and Line 36. A polymerase chain reaction (PCR) approach that amplified a sequence within the nucleotide binding site domain of the *N* gene yielded 143 NGHs, 106 of them translatable, that fell into 15 groups based on phylogenetic analyses. One of the groups was identified as the *N* gene itself. Further work is required to characterize this R gene family to identify the putative tombusvirus R gene sequence.

On the pathogen side, previous studies showed that the TBSV cell to cell movement protein P22 elicited HR in *N. glutinosa* and in its derived species *N. edwardsonii*, and the long distance movement protein P19 elicited HR in *N. tabacum*. We developed an agroinfiltration assay for transient expression of p22 and p19 genes, and found that *Nicotiana* species were able to recognize subtle differences between TBSV, CNV and CymRSV homologous P22 and P19 proteins in their role as Avr determinants. The three P19s were suppressors of gene silencing, but their suppressor function varied in strength and duration in *N. benthamiana*. We traced the resistance elicited by the P19 protein in *N. tabacum* to its ancestor *N. sylvestris*, but *N. tabacum* did not respond with HR to CNV P20.

Furthermore, we explored the genetic diversity in the *Nicotiana* genus against TBSV, CNV and CymRSV by inoculation of virions onto 18 *Nicotiana* species that belong to 10 out of 14 taxonomic sections. We found that 10 species showed HR, 5 were resistant without classical HR, and 3 were susceptible. We subsequently agroinfiltrated the three tombusvirus p22 and p19 genes into leaves of each of the 18 species, and we confirmed that *N. glutinosa* and *N. edwardsonii* responded to all three P22 variants with HR. In addition to *N. tabacum* and *N. sylvestris*, only *N. bonariensis* responded with HR to TBSV P19. Since many of the other *Nicotiana* species responded to TBSV virion inoculations with HR, we hypothesized that tombusvirus genes other than p22 and p19 could act as Avr determinants. To investigate this hypothesis, we agroinfiltrated the TBSV replicase genes p33 and p92, and the coat protein gene p41 into each of the *Nicotiana* species and found that *N. langsdorfii*, *N. bonariensis* and *N. longiflora*, all species of the Alatae section, responded to p41 with HR. The Alatae section contains an additional four species, so we hypothesized that these other 4 species would also respond to p41 with HR. In fact, we found that *N. alata*, *N. forgetiana*, and *N. mutabilis* were resistant to TBSV virions and recognized the p41 gene as Avr determinant. However, *N. plumbaginifolia* was susceptible to TBSV infection and agroinfiltration of p41 did

not trigger an HR. Based on these results we suggest that at least five types of R genes exist in *Nicotiana* species that can recognize TBSV determinants. One R gene recognizes P22 proteins in *N. glutinosa* (Undulatae section) and an accession of *N. forgetiana* (Alatae section). A second is able to recognize the P19 protein in *N. sylvestris* and in some species of Alatae. A third R gene in *N. tabacum* is a variant that can recognize TBSV P19 and CymRSV P19 proteins, but is unable to recognize the CNV P20. A fourth R gene is present in younger species of the Alatae section and recognizes the TBSV p41 gene as avirulence determinant. Finally, there still remains to be discovered at least one more R gene that is present in HR-resistant species that did not respond to individual agroinfiltration of TBSV genes. We showed that agroinfiltration is a powerful technique to screen not only a diversity of plant species as sources for R genes, but also to express viral genes as possible Avr determinants, and this strategy also has value for studying the evolution of resistance genes and their functionality across an entire genus.