Characterization of N gene homologs in Nicotiana species

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We have developed a new variety of Nicotiana, N. edwardsonii var. Columbia, that can be used as a bridge plant to move virus resistance genes from N. glutinosa to N. clevelandii, and have made crosses between N. edwardsonii var. Columbia and N. clevelandii to characterize a single dominant gene that specifies resistance to Tomato bushy stunt virus (TBSV). We have also developed a gene silencing assay that specifically targets host resistance genes that fall into the NBS-LRR category and have used this assay to show that TBSV resistance gene has sequence similarity to the N resistance gene. As a prelude to cloning the TBSV resistance gene, it is now important to understand how many N gene homologs exist in crosses with N. glutinosa. To examine the diversity of N gene homolog sequences, we developed PCR primers that amplified a 516 bp DNA segment of Exon 2 of the N gene. This PCR reaction yielded multiple products, which were cloned and sequenced. In N. clevelandii, two N homologs predominated. These sequences differed from the N gene by approximately 10%. The analysis of N homologs in N glutinosa is still being completed, but preliminary results indicate that this Nicotiana species contains a broader array of N gene homologs than N. clevelandii.