Introgression of a Tombusvirus Resistance Locus from *Nicotiana edwardsonii* var. Columbia to *N. clevelandii*

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ABSTRACT

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A new variety of *Nicotiana*, *N. edwardsonii* var. Columbia, was evaluated for its capacity to serve as a new source for virus resistance genes. Columbia was developed from a hybridization between *N. glutinosa* and *N. clevelandii*, the same parents used for the formation of the original *N. edwardsonii*. However, in contrast to the original *N. edwardsonii*, crosses between Columbia and either of its parents are fertile. Thus, the inheritance of virus resistance genes present in *N. glutinosa* could be characterized by using Columbia as a bridge plant in crosses with the susceptible parent, *N. clevelandii*. To determine how virus resistance genes would segregate in interspecific crosses between Columbia and *N. cleve-*

landii, we followed the fate of the *N* gene, a single dominant gene that specifies resistance to *Tobacco mosaic virus* (TMV). Our genetic evidence indicated that the entire chromosome containing the *N* gene was introgressed into *N. clevelandii* to create an addition line, designated *N. clevelandii* line 19. Although line 19 was homozygous for resistance to TMV, it remained susceptible to *Tomato bushy stunt virus* (TBSV) and *Cauliflower mosaic virus* (CaMV) strain W260, indicating that resistance to these viruses must reside on other *N. glutinosa* chromosomes. We also developed a second addition line, *N. clevelandii* line 36, which was homozygous for resistance to TBSV. Line 36 was susceptible to TMV and CaMV strain W260, but was resistant to other tombusviruses, including *Cucumber necrosis virus*, *Cymbidium ringspot virus*, *Lettuce necrotic stunt virus*, and *Carnation Italian ringspot virus*.

Throughout history, interspecific crosses have played a key role in the development of plant varieties that are resistant to pathogen attack. For example, the first virus resistance gene to be characterized was identified through an interspecific cross. Holmes (21) transferred the *N* gene, which conditions resistance to *Tobacco mosaic virus* (TMV), from *Nicotiana glutinosa* to *N. tabacum* through the use of the artificially produced *Nicotiana* species *N. digluta*, which was a spontaneously occurring amphiploid that resulted from a cross between *N. glutinosa* and *N. tabacum* (7). *N. digluta* was self fertile, contained the full chromosome complement from both *N. glutinosa* and *N. tabacum*, and could be successfully backcrossed to *N. tabacum* (6,14). The *N. tabacum* line created by Holmes, ‘Holmes Samsoun’, actually was a substitution line, in which the *N. glutinosa* chromosome containing the *N* gene replaced chromosome H of *N. tabacum* (12,13,21,22).

Although *N. digluta* is not commonly used today, other artificially produced *Nicotiana* species such as *N. edwardsonii* have been widely used and accepted. *N. edwardsonii* was synthesized from a cross between *N. glutinosa* and *N. clevelandii* parents nearly 40 years ago (3,4). *N. edwardsonii* is homozygous for the presence of the *N* gene, so it responds to TMV with a hypersensitive response (HR), but TMV infections frequently in-

duce the development of systemic necrosis symptoms, even in mature plants and at temperatures below 24°C (3,8). *N. edwardsonii* is also resistant to other viruses, including *Cauliflower mosaic virus* (CaMV) strain W260 (27) and the cherry strain of *Tomato bushy stunt virus* (TBSV) (30). However, it has not been possible to examine the inheritance of resistance to these viruses because the F1 hybrid between *N. edwardsonii* and *N. clevelandii* is infertile (9).

We recently resynthesized *N. edwardsonii* from its parents, *N. glutinosa* and *N. clevelandii* (9), and found that the hybrid we made differed in many aspects from the original *N. edwardsonii*, but most importantly, the progeny of crosses with either *N. glutinosa* or *N. clevelandii* were fertile. To distinguish this hybrid from the original *N. edwardsonii*, we designated our plant, *N. edwardsonii* var. Columbia (Columbia). Cole et al. (9) speculated that the enhanced fertility of Columbia might be related to the observation that it had the full complement of chromosomes from both *N. glutinosa* and *N. clevelandii* ($n = 72$), whereas two pairs of chromosomes were missing from the genome of the original *N. edwardsonii* ($n = 68$) (3). Consequently, we can use *N. edwardsonii* var. Columbia as a bridge plant to move resistance genes from *N. glutinosa* to *N. clevelandii* and characterize their inheritance, essentially the same strategy used by Francis Holmes in his genetic characterization of the *N* gene over 65 years ago (21).

In this paper, we present evidence that crosses between Columbia and *N. clevelandii* can be utilized to isolate pairs of *N. glutinosa* chromosomes in the susceptible *N. clevelandii* genotype. *N. clevelandii* addition lines can then be used to characterize virus resistance associated with a specific *N. glutinosa* chromosome pair. We have used this approach to characterize a new source of resistance that is effective against several tombusviruses.

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*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains supplemental material not included in the print edition. A supplemental figure illustrates mitotic metaphase chromosomes of *N. clevelandii* line 19 determined from a single cell.

MATERIALS AND METHODS

Viruses and plants. The synthesis of *N. edwardsonii* var. Columbia is described in Cole et al. (9). To break dormancy in hybrid seeds, they were treated with 2% (vol/vol) NaOCl for 30 min (2). For cytological analysis, root tips were prepared and analyzed according to Cole et al. (9). Three root tips of *N. cleve-landii* line 19 produced mitotic metaphase chromosomes and chromosome numbers were determined in five cells. All cells of line 19 contained 50 chromosomes. Six root tips of *N. cleve-landii* line 36 produced mitotic metaphase chromosomes and chromo-some numbers were determined in seven cells. All cells of line 36 contained 50 chromosomes.

For inoculations, *Nicotiana* leaves were lightly dusted with 600 mesh Carborundum and gently rubbed with an index finger dipped in the virus inoculum. TMV strain U1 inoculum was prepared from infected tobacco (*N. tabacum*) leaves by grinding them with a mortar and pestle and then diluting to approximately 1:20 (wt/vol) with inoculation buffer (0.05 M phosphate buffer, pH 7.0). In some tests, purified TMV virions were inoculated at a concentration of 50 µg/ml. CaMV strain W260 (15,27) was inoculated as partially purified virions, as described in Schoelz et al. (28). The tombusviruses TBSV, *Cucumber necrosis virus* (CNV), *Carnation Italian ringspot virus* (CIRV), and *Cymbidium ringspot virus* (CymRSV) have all been cloned in infectious form (16, 19,25,26). Infectious, uncapped transcripts derived from the clones were made according to Scholthof et al. (29,31) and initially inoculated to either *N. cleve-landii* or *N. benthamiana*. *Lettuce necrotic stunt virus* (LNSV) strain L2 (23) was main-tained in *N. benthamiana*. For inoculation of test plants, leaves infected with tombusviruses were ground with a mortar and pestle and diluted to approximately 1:20 (wt/vol) with inoculation buffer. For the host range evaluation of *N. cleve-landii* lines 19 and 36, at least 15 plants were inoculated with each virus and observed up to 21 days for symptoms. Virus inoculations were conducted in the greenhouse during the months of September through May, and in growth chambers set at 22°C during the months of June through August.

Alignment of P22 amino acid sequences. The tombusvirus P22 protein sequences were aligned with ClustalW, a program obtained from the European Bioinformatics Institute (Cambridge, UK). The database accession numbers for tombusvirus P22 proteins are as follows: TBSV-cherry isolate (EMBL M31019) (19), CIRV (EMBL X85215) (26), CNV (EMBL M25270) (25), CymRSV (EMBL X15511) (16), and LNSV strain L2 (NCBI DQ238848) (23).

Polymerase chain reaction detection of the *N* gene. Total DNA was isolated from *Nicotiana* species according to Dellaporta et al. (10). Primer sequences for PCR amplification of the *N* gene were derived from the published *N* gene sequence (33) and were synthesized by Integrated DNA Technologies (Coralville, IA). The forward *N* gene primer corresponded to nucleotides 2707 (5'-GGCAATGGAAGCAATTGGGT-3') to 2727, and the reverse

primer corresponded to nucleotides 3601 (5'-CTATAATTTGTA-TATTAAGTT-3') to 3581 (33). Polymerase chain reaction (PCR) conditions were 94°C for 5 min, 55°C for 30 s, and 72°C for 2 min, for 1 cycle, followed by 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, for 35 cycles.

RESULTS

Introgression of the *N* gene chromosome from *N. edward-sonii* var. Columbia to *N. cleve-landii*. We chose to first examine the inheritance of the *N* gene because it could serve as a guide for how a single dominant gene would segregate in this interspecific cross. *N. cleve-landii* develops a systemic yellowing after inoculation with TMV, whereas *N. glutinosa* and Columbia respond to TMV with HR; both *N. glutinosa* and Columbia carry the *N* gene. As expected, all of the F1 hybrid plants (*N. cleve-landii* × Colum-bia) also developed an HR 2 days after inoculation with TMV (Table 1). In successive backcrosses with *N. cleve-landii* as the re-current parent, the plants that responded with HR to TMV were readily distinguished from susceptible siblings (Fig. 1A). Al-though a segregation ratio of 1:1 might have been expected in the B1 generation, only 13 of 63 plants responded with HR (Table 1), an indication that the *N* gene was being carried forward to the B1 generation on a single, unpaired chromosome. Subsequent back-crosses approached a segregation ratio of 1:1 for the *N* gene; how-ever, only 30 of 80 plants of the "B3 Self" generation responded to TMV with HR, an indication that even in the B3 generation the *N* gene existed on a single, unpaired chromosome.

If the *N* gene chromosome existed as an unpaired chromosome in the B3 generation, then some of the progeny that resulted from self-pollination of a B3 plant might be expected to acquire this chromosome from both the pollen and ovule; these plants would be homozygous for the *N* gene. To examine this possibility, each of the "B3 Self" plants that responded with HR to TMV was self pollinated, and approximately 20 progeny were screened for their response to TMV. Of the 23 families screened, 19 exhibited segre-gation for resistance to TMV, whereas four families were homo-zygous for resistance. We determined the chromosome number in one of the families homozygous for TMV resistance, designated *N. cleve-landii* line 19, and found that it contained 50 chromo-somes (data not shown). Since *N. cleve-landii* contains 48 chromo-somes, we propose that *N. cleve-landii* line 19 is an addition line; the *N. glutinosa* chromosome pair that contains the *N* gene has been incorporated into *N. cleve-landii*. PCR with primers specific for the *N* gene confirmed that line 19 had indeed acquired the *N* gene (Fig. 2). An 894-bp PCR band was present in *N. glutinosa*, Columbia, and line 19, but absent from *N. cleve-landii*.

The creation of *N. cleve-landii* line 19 presented an opportunity to examine whether resistance to other viruses might cosegregate with the *N. glutinosa* chromosome that contains the *N* gene. For example, other viruses besides TMV are able to elicit HR in *N. edwardsonii*, viruses such as CaMV strain W260 (27) and TBSV (30). However, both of these viruses were able to systemically infect line 19 (Table 2), indicating that resistance genes against these viruses were located on other *N. glutinosa* chromosomes.

Introgression of a tombusvirus resistance locus from *N. edwardsonii* var. Columbia to *N. cleve-landii*. The inheritance patterns of the *N* gene in crosses between Columbia and *N. cleve-landii* indicated that it should be possible to genetically characterize resistance to other viruses such as TBSV. When TBSV was inoculated to F1 plants of the cross, *N. cleve-landii* × Columbia, all plants responded with HR (Table 3), an indication that resistance was dominant. In successive backcrosses with *N. cleve-landii* as the recurrent parent, the plants that responded with HR to TBSV were readily distinguished from susceptible siblings because they responded with HR at 2 to 3 days post-inoculation (dpi) (Fig. 1B). Plants that acquired the TBSV resis-tance gene did not develop any symptoms on upper, noninocu-

TABLE 1. Selection of *Nicotiana* hybrids that respond to Tobacco mosaic virus (TMV) with hypersensitive response (HR)

Host	Response to TMV inoculation		χ^2
	Observed ratio HR:susceptible	Expected ratio HR:susceptible	
<i>N. cleve-landii</i>	0:11	...	...
<i>N. edwardsonii</i> var. Columbia	13:0	...	...
F1 (<i>N. cleve-landii</i> × Columbia)	5:0	...	...
B1 (F1 × <i>N. cleve-landii</i>)	13:50	31.5:31.5	21.70 ^a
B2 (B1 × <i>N. cleve-landii</i>)	11:9	10:10	0.20
B3 (B2 × <i>N. cleve-landii</i>)	6:10	8:8	1.00
B3 self	30:50	60:20	60.00 ^a

^a Significant deviation ($P = 0.05$) from the expected ratio.

lated leaves and these leaves remained free of the virus, as assessed by enzyme-linked immunosorbent assay (data not shown). In contrast, siblings that did not acquire the TBSV resistance gene developed systemic necrosis and were killed by 25 dpi. Although results obtained with the F1 generation were indicative of a dominant gene, the results obtained in backcross generations B2 through B4 did not approximate the 1:1 segregation ratio expected for a single, dominant gene (Table 3). For example, in the B3 generation, only 20 of 80 plants inoculated responded with HR. Furthermore, the segregation ratios in the “B3 Self” generation also did not approximate a 3:1 segregation ratio expected for a single, dominant gene.

As with the *N* gene, an alternative explanation for the aberrant segregation ratios might be that the TBSV resistance gene was

being carried forward into each of the backcross generations on a single, unpaired *N. glutinosa* chromosome. To examine this possibility, each of the “B3 Self” plants that responded with HR to TBSV was self-pollinated, and approximately 20 progeny screened for their response to TBSV. Of 60 families screened, 59 exhibited segregation for resistance to TBSV, whereas one family was homozygous for resistance. The resistance response of this family, designated *N. clevelandii* line 36, to inoculation with TBSV is illustrated in Figure 1C. In contrast to line 36, the wild-type *N. clevelandii* plants that were inoculated with TBSV at the same time were nearly dead by 20 dpi (Fig. 1D). We determined the chromosome number in line 36 and found that it contained 50 chromosomes (data not shown). As with *N. clevelandii* line 19, we propose that line 36 is an addition line, in which a second pair

Fig. 1. Response of *Nicotiana* hybrids to *Tobacco mosaic virus* (TMV) and *Tomato bushy stunt virus* (TBSV). **A**, Selection of “Backcross 2” plants that respond to inoculation with TMV with hypersensitive response (HR). The red arrows illustrate the inoculated leaves that responded with HR and have since died. **B**, Selection of “Backcross 2” plants that respond to inoculation with TBSV with HR. **C**, Response of *N. clevelandii* line 36 plants to TBSV at 20 days postinoculation (dpi). **D**, Response of *N. clevelandii* plants to TBSV at 20 dpi. **E**, Response of *N. clevelandii* line 36 plants to *Cucumber necrosis virus* (CNV) at 20 dpi. **F**, Response of *N. clevelandii* plants to CNV at 20 dpi. The photos in panels **C** to **F** were all taken on the same day.

of *N. glutinosa* chromosomes have been incorporated into the *N. clevelandii* genome.

Other tobamoviruses in addition to TBSV are able to elicit HR in *N. edwardsonii*, viruses such as CymRSV, CNV, CIRV, and LNSV. Line 36 responded with HR and resistance to each of these tobamoviruses (Table 2), whereas *N. clevelandii* developed a systemic yellowing that progressed into a cell death symptom. The responses of line 36 and *N. clevelandii* to CNV at 20 dpi are illustrated in Figure 1E and F. Although each of the tobamoviruses elicited HR, the intensity of the HR was not equivalent for all viruses. TBSV, CNV, and LNSV elicited large necrotic lesions by 2 dpi, whereas CymRSV and CIRV elicited smaller necrotic lesions that appeared approximately 1 day later. The resistance in line 36 was specific to the tobamoviruses, as line 36 was susceptible to TMV and CaMV strain W260 (Table 2).

DISCUSSION

In this paper, we have characterized a new resistance locus that is effective against five distinct tobamoviruses. The key to the characterization of this locus was a new variety of *N. edwardsonii*, *N. edwardsonii* var. Columbia (9), which could be used as a bridge plant to move resistance genes from *N. glutinosa* into *N. clevelandii*. To investigate whether Columbia could be used as a source for new resistance genes, we first examined how the *N* gene would segregate in repeated backcrosses to *N. clevelandii*. We found that unpaired *N. glutinosa* chromosomes tended to be carried from one generation to the next. In fact, Goodspeed (14) noted that this would be the expected outcome of many crosses between species of the genus *Nicotiana*. The end result of these crosses was *N. clevelandii* line 19. Our cytological and genetic evidence indicated that line 19 was an addition line that had the full complement of *N. clevelandii* chromosomes, plus the pair of *N. glutinosa* chromosomes that carried the *N* gene. *N. clevelandii* line 19 consistently responded to TMV inoculation with HR and its genome contained 50 chromosomes.

The development of line 19 created two opportunities for the discovery of other virus resistance genes. First, we could deter-

mine whether the *N. glutinosa* chromosomes present on line 19 carried dominant resistance alleles to other viruses. Resistance genes frequently occur in clusters (17,24), and the *N* gene is thought to exist within a cluster of related resistance gene sequences (33). The two viruses that we tested, CaMV strain W260 and the cherry strain of TBSV, systemically infected line 19 plants, but elicited HR in both Columbia and *N. edwardsonii* (27,30). A previous study had shown that resistance to CaMV strain W260 in Columbia is dominant (9), and we found in the present study that resistance to TBSV is also dominant. Consequently, we concluded that resistance to both of these viruses must reside on a different *N. glutinosa* chromosome, or must be conditioned by more than one gene. Although line 19 plants were not resistant to either CaMV strain W260 or TBSV, line 19 may still serve as a resource to determine if other dominant virus resistance genes are located on the same *N. glutinosa* chromosome as the *N* gene.

The creation of *N. clevelandii* line 19 was valuable for a second reason, because it illustrated how other single dominant resistance genes might be expected to segregate in backcrosses with *N. clevelandii*. Consequently, we were able to develop a second addition line, *N. clevelandii* line 36, which was homozygous for resistance to the cherry strain of TBSV, as well as resistance to CymRSV, CNV, LNSV, and CIRV. Since line 36 was susceptible to TMV, we believe that we have introgressed a second pair of *N. glutinosa* chromosomes into an *N. clevelandii* background. At this point, we cannot state whether a single plant gene conditions resistance to all five tobamoviruses, or whether multiple resistance genes exist on the same *N. glutinosa* chromosome, either as a cluster or scattered throughout its length.

The target for the TBSV resistance gene is P22, a viral protein necessary for cell-to-cell movement (31). A detailed genetic analysis of the P22 protein has shown that the cell-to-cell movement function can be separated from sequences that elicit HR (5). In particular, the mutations in P22 located at amino acids 14, 18, and 59 were able to abolish HR in *N. edwardsonii*, as these mutants elicited chlorotic lesions in *N. edwardsonii* and were competent for both cell-to-cell and long-distance movement. Chu et al. (5) suggested that *N. edwardsonii* must contain host factors capable of specific recognition of the TBSV P22 protein that

Fig. 2. Amplification of a portion of Exon 3 of the *N* gene from *Nicotiana glutinosa* (Ng), *N. edwardsonii* var. Columbia (NeC), and *N. clevelandii* line 19 (19), but not from *N. clevelandii* (Nc). The sequences amplified correspond to nucleotides 2707 to 3601.

TABLE 3. Selection of *Nicotiana* hybrids that respond to *Tomato bushy stunt virus* (TBSV) with hypersensitive response (HR)

Host	Response to TBSV inoculation		
	Observed ratio HR:susceptible	Expected ratio HR:susceptible	χ^2
<i>N. clevelandii</i>	0:12	...	...
<i>N. edwardsonii</i> var. Columbia	12:0	...	...
F1 (<i>N. clevelandii</i> $\times$ Columbia)	8:0	...	...
B1 (F1 $\times$ <i>N. clevelandii</i>)	2:3	...	...
B2 (B1 $\times$ <i>N. clevelandii</i>)	11:34	22.5:22.5	11.8 ^a
B3 (B2 $\times$ <i>N. clevelandii</i>)	20:60	40:40	20.0 ^a
B4 (B3 $\times$ <i>N. clevelandii</i>)	24:53	38.5:38.5	10.9 ^a
B3 self	61:42	77.25:25.75	13.7 ^a

^a Significant deviation ($P = 0.05$) from the expected ratio.

TABLE 2. Response of *Nicotiana* species and addition lines to inoculation with selected viruses

Virus	Response ^a			
	<i>N. edwardsonii</i> var. Columbia	<i>N. clevelandii</i>	<i>N. clevelandii</i> line 19	<i>N. clevelandii</i> line 36
Tobacco mosaic virus	HR	SY	HR	SY
Cauliflower mosaic virus strain W260	HR	SN	SN	SM
Tomato bushy stunt virus cherry strain	HR	SN	SN	HR
Cucumber necrosis virus	HR	SN	nt	HR
Carnation Italian ringspot virus	HR	SN	nt	HR
Cymbidium ringspot virus	HR	SN	nt	HR
Lettuce necrotic stunt virus strain L2	HR	SN	nt	HR

^a HR, hypersensitive response, no systemic movement; SY, systemic yellowing; SN, systemic necrosis; SM, systemic mosaic; and nt, not tested.

would result in activation of plant defenses. Furthermore, these host factors might be unrelated to those that interact with the P22 protein to facilitate movement. One host protein has been identified that interacts with the P22 protein, a plant homeodomain protein, but it is not known if it mediates the host defense response (11).

Our present study provides genetic evidence for a host resistance gene that is able to recognize the TBSV P22 protein. Although it is not yet known if the resistance to CymRSV, CIRV, CNV, and LNSV also targets each of their P22 proteins, there is a significant degree of homology between each of these proteins and TBSV P22 (Fig. 3). In particular, TBSV amino acids previously shown to be involved in elicitation of HR (amino acids 14, 18, and 59) (5) are conserved in the P22 proteins of the tomosviruses in this study. Consequently, a single host gene might recognize the P22 of divergent tomosviruses, although this must be proven.

Tomosviruses cause economically important diseases in tomato, eggplant, and lettuce worldwide (23), but there are few examples in the literature of tomosvirus resistance genes. Recently, a single dominant gene was found in lettuce that confers resistance to LNSV and TBSV, and this gene, *Tvr1*, is considered to be the first example of resistance to any tomosvirus (18). It is not known if *Tvr1* is effective against other tomosviruses, or which tomosvirus protein it targets. The resistance we have characterized in *N. glutinosa* could be valuable, as it could provide a source of resistance to a broad range of tomosviruses, regardless of whether a single gene targets all tomosviruses or multiple resistance genes target specific tomosviruses.

Nicotiana species can be a valuable source for resistance genes against pathogens that affect solanaceous crops. For example, Heist et al. (20) recently found that *N. obtusifolia* responds with HR to *Peronospora tabacina*, the causal agent of blue mold of tobacco. Our study demonstrates the utility of using Columbia as a bridge plant to move resistance genes from *N. glutinosa* to *N. clevelandii*. Beyond TBSV and TMV resistance, Columbia could be of value for isolation of resistance genes to a large number of plant viruses. By cross referencing information in published compilations (1), we can identify 67 plant viruses distributed amongst 20 genera that have been shown to infect *N. clevelandii*, but cannot infect *N. glutinosa* (Table 4). Several of the viruses on the list have been well characterized at the molecular level and some cause severe economic losses (32). It remains to be seen if resistance to these viruses is determined by single or multiple genes, but Columbia may serve as a useful model host to explain on a molecular level how a single plant can recognize and defend against a broad range of viruses.

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TBSV	MDTEYEQVKNKPWN ELYKE ETTLGNKLTVNVGMEDQEVPLLP SNFLT KVRVGLSGGYITM RR	60
CIRV	MDTEYEQVKNKPWN ELYKE ETTLGNKLTVNVGMEDQEVSLLP SNFLT KVRVGLSGGYITM RR	60
LNSV	MDTEYEQVKNKPWN ELYKE ETTLGNKLTVNVGMEDQEVALLP SNFLT KVRVGLSGGYITM RR	60
CNV	MDTEYEQVKNKPWN ELYKE ATLGNKLTVNVG MEDVEVPLLP SNFLT KVRVSLSGGYITV RR	60
CymRSV	MDTEYQVKNKPWN ELYKE VT LGNKLTVNVGMEEEVLLLP SNFP TKVRVSMGGYITV RR	60

TBSV	IRIKIIP LVS RKAGVSGKLYLRDISDTTGRKLHCTESLDL GREIRLTMQH LDFS VSTRSD	120
CIRV	IRIKIIP LVS RKAGVSGKLYLRDISDTTGRKLHCTESLDL GREIRLTMQH LDFS VPTRSD	120
LNSV	IRIRITP LVS RKAGVSGKLYLRDISDTTGRKLHCTESLDL GREIRLTMQH LDFS VSTRSD	120
CNV	VRIKIIP LVS RKAGVSGKLYLRDISDTTGRKLHCTE LLDLGKEIRLTMQH LDFS VSARS D	120
CymRSV	VRIRIIP LVS RKAGVSGKLYLRDISDTTGQKLHCTE LLDLGKEIRLTM PH LDFS VSAKSD	120
	** *	
TBSV	VPIVFGFEELVSPFLEGRELFSISVRWQFGLSKNCYSLPQSKWKVMYQEDALKVLRPSK-	179
CIRV	VPIVFGFEELVSPFLEGRELFSISVRWQFGLSKNCYSLPQSKWKVMYQEDALKVLRPSK-	179
LNSV	VPIVFGFEELVSPFLEGRELFSISVRWQFGLSKNCYSLPQSKWKVMYQEDALKVLRPSK-	179
CNV	VPIVFGFEDLVSPYLEGRELF SVSLRWQFGLSAQCYS LPPAKWKVMYQEDALKALKPSKI	180
CymRSV	VP IAFGFEELVSPFREGRELFSVSLRWQLGLSAQCYS LPPANVKVMYQEDALKALKPSK-	179

TBSV	KKASKTDSSV	189
CIRV	KKASKTDSSV	189
LNSV	KKASKTDSSV	189
CNV	KKASKTDSSV	190
CymRSV	KKASRTDSSV	189

Fig. 3. Alignment of the amino acid sequences of the p22 proteins of *Tomato bushy stunt virus* (TBSV), *Cucumber necrosis virus* (CNV), *Carnation Italian ringspot virus* (CIRV), *Lettuce necrotic stunt virus* (LNSV), and *Cymbidium ringspot virus* (CymRSV). Asterisks indicate sequence identity across all four sequences. The amino acids, at positions 14, 18, and 59, are underlined and printed in bold. They indicate residues that have been shown to be involved in elicitation of hypersensitive response.

TABLE 4. Sixty-seven viruses reported to infect *Nicotiana clelandii* but not *N. glutinosa*

Virus/Family	Virus/Family	Virus/Family
Potyvirus	Tombusvirus	Potexvirus
<i>Alstroemeria mosaic virus</i>	<i>Carnation Italian ringspot virus</i>	<i>Dioscorea latent virus</i>
<i>Amaranthus leaf mottle virus</i>	<i>Cucumber necrosis virus</i>	<i>Lily X virus</i>
<i>Asystasia gangetica mottle virus</i>	<i>Cymbidium ringspot virus</i>	<i>Narcissus mosaic virus</i>
<i>Bean common mosaic virus</i>	<i>Eggplant mottled crinkle virus</i>	<i>Plantain X virus</i>
<i>Beet mosaic virus</i>	<i>Lettuce necrotic stunt virus</i>	
<i>Bidens mottle virus</i>	<i>Pelargonium ringspot virus</i>	Ourmiavirus
<i>Carrot thin leaf virus</i>	<i>Pepper Moroccan virus</i>	<i>Epirus cherry virus</i>
<i>Chickpea filiform virus</i>	<i>Tomato bushy stunt virus</i>	<i>Olive latent 2 virus</i>
<i>Clover yellow vein virus</i>		
<i>Daphne Y virus</i>	Tymovirus	Vitivirus
<i>Lettuce mosaic virus</i>	<i>Clitoria yellow vein virus</i>	<i>Grapevine A virus</i>
<i>Passionfruit woodiness virus</i>	<i>Okra mosaic virus</i>	
<i>Peanut green mosaic virus</i>	<i>Ononis yellow mosaic virus</i>	Nepovirus
<i>Peanut mottle virus</i>		<i>Grapevine fanleaf virus</i>
<i>Pepper vein mottle virus</i>	Carmovirus	<i>Olive latent ringspot virus</i>
<i>Poplar decline virus</i>	<i>Carnation mottle virus</i>	<i>Tomato ringspot virus</i>
<i>Statice Y virus</i>	<i>Cucumber leaf spot virus</i>	
<i>Sweet potato latent virus</i>		Tobamovirus
<i>Telfairia mosaic virus</i>	Closterovirus	<i>Tobacco mosaic virus</i>
<i>Ullucus mosaic virus</i>	<i>Carrot yellow leaf virus</i>	
<i>Vallota mosaic virus</i>	<i>Cassia severe mosaic virus</i>	Umbravirus
<i>Vanilla necrosis virus</i>		<i>Groundnut rosette virus</i>
<i>Watermelon mosaic 2 virus</i>	Carlavirus	
<i>Wisteria vein mosaic virus</i>	<i>Cowpea mild mottle virus</i>	Iarvirus
	<i>Hop mosaic virus</i>	<i>Humulus japonicus virus</i>
Tobravirus	<i>Nerine latent virus</i>	<i>Hydrangea mosaic virus</i>
<i>Pea early browning virus</i>		<i>Parietaria mottle virus</i>
	Comovirus	
Tospovirus	<i>Cowpea mosaic virus</i>	Caulimovirus
<i>Tomato spotted wilt virus</i>	<i>Radish mosaic virus</i>	<i>Cauliflower mosaic virus</i>
	<i>Ullucus C virus</i>	
Bromovirus	Furovirus	Sobemovirus
<i>Broad bean mottle virus</i>	<i>Broad bean necrosis virus</i>	<i>Lucerne transient streak virus</i>
<i>Cowpea chlorotic mottle virus</i>		

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