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Identification and Map Location of *TTR1*, a Single Locus in *Arabidopsis thaliana* that Confers Tolerance to Tobacco Ringspot Nepovirus

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The interaction between *Arabidopsis* and the nepovirus tobacco ringspot virus (TRSV) was characterized. Of 97 *Arabidopsis* lines tested, all were susceptible when inoculated with TRSV grape strain. Even though there was systemic spread of the virus, there was a large degree of variation in symptoms as the most sensitive lines died 10 days after inoculation, while the most tolerant lines either were symptomless or developed only mild symptoms. Four lines were selected for further study based on their differential reactions to TRSV. Infected plants of line Col-0 and Col-0 *gl1* flowered and produced seeds like noninfected plants, while those of lines Estland and H55 died before producing seeds. Symptoms appeared on sensitive plants approximately 5 to 6 days after inoculation. Serological studies indicated that in mechanically inoculated seedlings, the virus, as measured by coat protein accumulation, developed at essentially the same rates and to the same levels in each of the four lines, demonstrating that differences in symptom development were not due to a suppression of virus accumulation. Two additional TRSV strains gave similar results when inoculated on the four lines. Genetic studies with these four *Arabidopsis* lines revealed segregation of a single incompletely dominant locus controlling tolerance to TRSV grape strain. We have designated this locus *TTR1*. By using SSLP and CAPS markers, *TTR1* was mapped to chromosome V near the nga129 marker. Seed transmission frequency of TRSV for Col-0 and Col-0 *gl1* was over 95% and their progeny from crosses all had seed transmission frequencies of over 83%, which made it possible to evaluate the segregation of *TTR1* in F_2 progeny from infected F_1 plants without inoculating F_2 plants. Seed transmission of TRSV will be further exploited to streamline selection of individuals for fine mapping the *TTR1* gene. The identification of tolerant and sensitive interactions between TRSV and *A. thaliana* lines provides a model system for genetic and molecular analysis of plant tolerance to virus infection.

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Tobacco ringspot virus (TRSV) is the type member of the nepovirus group of plant viruses. Nepovirus genomes are bipartite, consisting of two single-stranded positive-sense polyadenylated RNA molecules (Murant et al. 1981). Some TRSV strains have satellite RNA associated with their genomes (Schneider et al. 1972). The satellite RNAs of TRSV either decrease or have little effect on the severity of symptoms caused by TRSV (Buzayan et al. 1986; Gerlach et al. 1986). TRSV is widespread and infects both dicotyledonous and monocotyledonous plants (Stace-Smith 1985). For soybeans, the virus is transmitted primarily by nematodes (Bergeson et al. 1964) and seeds (Athow and Bancroft 1959). The virus can cause economically important diseases in such diverse crops as blueberry (*Vaccinium* sp.), grapes (*Vitis* sp.), soybeans (*Glycine max*), and tobacco (*Nicotiana tabacum*) (Stace-Smith and Ramsdell 1987).

Arabidopsis thaliana (L.) Heynh. has been widely used as a model plant system for the study of plant-pathogen interactions including the identification of disease resistance genes active against bacteria, fungi, nematodes, and viruses (Simon 1994; Crute et al. 1994; Sijmons et al. 1994; Dangl 1995; Kunkel 1996). A hypersensitive response (HR) has been reported for some *Arabidopsis* lines infected with beet curly top virus (Lee et al. 1994), cauliflower mosaic virus (Leisner et al. 1993), cucumber mosaic virus (Takahashi et al. 1994), and turnip crinkle virus (Dempsey et al. 1993). A single resistance locus conferring a HR to beet curly top virus was identified in some *Arabidopsis* lines (Lee et al. 1994).

With the recent isolation of plant disease resistance genes (Dangl 1995; Staskawicz et al. 1995), the molecular basis for the "gene-for-gene" model (Flor 1947) of host-pathogen interactions has been further defined. In this model, the pathogen usually produces an avirulence factor that elicits a resistant response if the host carries a resistance gene of appropriate specificity. Gene-for-gene resistance is often associated with a HR (Keen 1990). If there is an absence of pathogen recognition by the host either due to the lack of a resistance gene in the host or the lack of an avirulence gene in the pathogen, a resistance response is not induced, and the infection proceeds toward disease. Resistance associated with a HR has been reported for many plant hosts to a variety of plant pathogens; however, not all host-pathogen interactions fit neatly into the gene-for-gene model.

Many plant-pathogen interactions lead to a host response termed tolerance. Tolerance and sensitivity have been used to describe distinctive responses of plants to pathogen infection (Mussel 1980; Cooper and Jones 1983). Tolerance occurs when plants are susceptible to infection by a pathogen, but the infection causes only mild or no symptoms. Plants are termed sensitive to infection when the infection causes conspicuous symptoms severely reducing the growth of the plant (Mussel 1980; Cooper and Jones 1983). Disease tolerance and disease resistance are not mutually exclusive, and plant breeders often select for both traits. Tolerance can be present in the absence of gene-for-gene resistance, and one highly desirable attribute of tolerance is its tendency to be effective across a broad range of pathogen strains or races.

Tolerance of plants to abiotic stresses has been extensively studied (Minorsky 1989; Chandler and Robertson 1994; Thomashow 1994; Vierling 1991). ABA, ethylene, and Ca^{2+} have been proposed to mediate the tolerance response of plants to these kinds of environmental stresses (Chen et al. 1983; Mattoo and Suttle 1991; Urao et al. 1994). While tolerance responses of plants to pathogen infection have not been clearly defined at the molecular level, Bent et al. (1992) associated mutations that cause reduced sensitivity to ethylene with tolerance of *Arabidopsis* to infection by bacterial pathogens. To understand the mechanism of tolerance to plant pathogens, host factors conferring tolerance must be cloned and analyzed. Such an effort is in progress for *RXC1*, a gene that controls tolerance of *Arabidopsis* to infection by *Xanthomonas campestris* pv. *campestris* (Tsuji et al. 1991; Buell and Somerville 1995). In addition, Zamir et al. (1994) recently mapped the chromosomal location of a gene in tomato, *TY-1*, that confers tolerance to tomato yellow leaf curl virus.

In this paper, we describe the interaction between TRSV and *Arabidopsis*. A tolerance response to TRSV infection in specific lines of *Arabidopsis* is reported. All of the *Arabidopsis* lines screened became systemically infected by TRSV upon inoculation. Both the tolerant and sensitive plants accumulated virus to essentially the same level, yet the tolerant plants were symptomless, while the sensitive plants became necrotic after inoculation. A single locus controlling the tolerance phenotype was identified and named *TTR1*. *TTR1* was mapped to chromosome V near marker nga129.

Table 1. Infectivity and symptom development in selected *Arabidopsis thaliana* lines inoculated with tobacco ringsot virus (TRSV) grape strain

Common name	Response	Common name	Response	Common name	Response
Aa-0	T[a]	DijonG	T	Ke-0	T
Ak-1	T	Do-0	T	Km-0	T
An-1	T	Edi-0	T	LD-2	T
Ang-0	T	Ei-4	T	La-0	T
Bay-0	T	En-1	T	La-1	T
Bch-4	T	Ep-0	T	Lan-0	T
Bd-0	T	Er-0	T	Lc-0	S, Chl
Be-1	T	Ler-0	T	Li-3	T
Ber	T	Es-0	T	Lip-0	T
Bl-1	T	Estland	S, Chl[e]	LI-0	T
Bla-3	T	Fe-1	T	Lz-0	T
Bs-2	T	Fi-0	T	Ma-0	T
Bsch-0	S[b], Stu[c]	Fi-1	T	Mnz-0	T
Bu-0	T	Fr-3	T	Mrk-0	T
Bur-0	S, Stu	Ga-2	T	Mt-0	T
C24	T	Ge-1	T	Mz-0	T
Cal-0	S, Stu	Gie-0	T	Nc-1	T
Ceo-0	T	Go-2	T	Nd-0	T
Chi-1	T	Gr-3	T, YM[f]	Nie-0	T
Cl-0	T	Gu-1	T	Np-0	S, Chl
Co-2	S, Stu	H55	S, Stu	Petergof	T
Col-0	T	Ha-0	S, Stu	Po-1	T
Col-2	T	Hh-0	T	Rld1	T
Col-3	T	Hl-2	T	Rubezhnoe-1	T
Col-4	T, Fl[d]	Ho-0	T	S96	T
Condara	T	Hodja-Obi-Gaz	T	Shahdara	T
CS2360	T	In-0	T	Sn(5)-1	T
Ct-1	T	Je-0	T	Sorbo	T
Cvi-0	T, Fl	Je54	T	WS-1	T
Da(1)-12	T	JI-1	T	Wei-0	T
Da-0	T	Ka-0	T	Ws-1	T
Db-2	T	Kl-1	T		
Di-2	T	Kn-0	T		

[a] Tolerant.
[b] Sensitive.
[c] Stunting.
[d] Flower inhibition.
[e] Chlorosis.
[f] Yellow mosaic.

RESULTS

Virulence of TRSV strains on *A. thaliana*.

Ninety-seven *Arabidopsis* lines were inoculated with TRSV grape strain. After 7 days, young noninoculated rosette leaves were ground in PBS and plant extracts were tested for the presence of TRSV by both ELISA and back inoculation of a sensitive soybean cultivar (cv. Williams). All 97 lines were shown to be infected by TRSV. Mock-inoculated plants did not transmit TRSV to soybean plants and had enzyme-linked immunosorbent assay (ELISA) readings not significantly above background. Most lines were tolerant to TRSV and developed only mild symptoms or were symptomless after in-

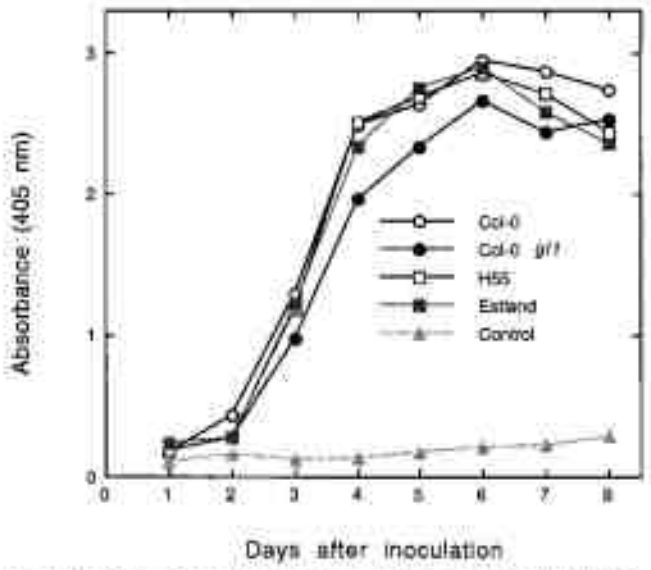

Fig. 1. Time course of coat protein accumulation in Col-0, Col-0 *gl*, H55 and Estland. Noninoculated upper leaves from Col-0, Col-0 *gl1*, H55 and Estland were collected and ground in PBS buffer. The coat protein in inoculated plants was detected by ELISA. The average amount of the coat protein in two independent ELISA test is shown by the absorbance at 405 nm.

oculation with TRSV grape strain (Table 1). Nine lines were sensitive to infection and exhibited severe symptoms that were clearly distinct from those observed on the tolerant lines such as Col-0 and Landsberg. The nine sensitive lines developed similar symptoms after infection with either the grape or bud blight strains of TRSV, although the grape strain caused more rapid symptom development in all sensitive lines compared with the bud blight strain. Viral RNA purified from soybean plants infected with the grape strain contained less associated satellite RNA than the bud blight strain (data not shown), which may account for the differences in observed symptoms. Based on their differential responses to TRSV, the two sensitive *Arabidopsis* lines H55 (cs6184) and Estland (cs6173) were selected for further study together with the tolerant line Columbia (Col-0).

TRSV caused either no visible symptoms or mild symptoms on plants of line Col-0 or on its glabrous isoline (Col-0 *gl1*). Severe symptoms occurred on plants of lines Estland and H55. Disease symptoms were first observed on newly emerging leaves. H55 plants were severely stunted while Estland plants were chlorotic 5 to 6 days after inoculation with TRSV grape strain. By 10 days after inoculation, all plants of lines Estland and H55 died without producing seeds. In contrast, infected plants of line Col-0 and Col-0 *gl1* grew, flowered, and produced seed like noninfected plants.

Viral coat protein accumulation in sensitive and tolerant lines.

To determine if virus accumulation as measured by coat protein was correlated with symptom development, mechanically inoculated plants were analyzed by ELISA. Thirty-two plants each of H55, Estland, Col-0, and Col-0 *gl1* were inoculated and grown under the same conditions. The accumulation of viral coat protein in noninoculated upper leaves was monitored over time using ELISA. The viral coat protein accumulated essentially at the same rate and to the same levels in each of the lines until 8 days after inoculation (Fig. 1). After 8 days, the sensitive lines were nearly dead and the virus concentration could no longer be reliably determined.

Genetics of TRSV tolerance.

The number of loci controlling Col-0 tolerance to TRSV infection was determined using a series of reciprocal crosses

Table 2. Segregation ratio of F_2 plants of Estland × Col-0 and Col-0 *gl1* × H55 to tobacco ringspot virus (TRSV) infection[a]

Cross[b]	Tolerant	IM[c]	Sensitive	χ^{2d}	P
Col-0 × Est	29	60	24	0.88	0.65
Est × Col	37	74	29	1.37	0.50
Col *gl* × H55	22	42	16	1.10	0.58
H55 × Col-0	29	52	22	0.96	0.62

[a] Data are presented from one of each pair of reciprocal crosses.
[b] The female parent is listed first for all crosses. F_2 plants were inoculated at four- to five-leaf stage. Symptom evaluation and ELISA were done 8 days after inoculation with TRSV grape strain for crosses Col-0 × Est and Col *gl* × H55, while data from Est × Col and H55 × Col-0 are for plants inoculated by seed transmission of the virus.
[c] IM: intermediate symptom, including leaf curling for Col × H55 and stunting and chlorosis for Est × Col.
[d] The χ^2 and corresponding P values were calculated to test the probability that the data obtained fit an expected 1:2:1 segregation for a single incomplete dominant locus that confers tolerance to TRSV grape strain.

Fig. 2. Symptoms of Col-0, Estland and the F_1 and F_2 plants to tobacco ringspot virus (TRSV) infection 10 days after inoculation. **A**, Col-0 (tolerant parent) without symptoms; **B**, F_1 of Estland × Col-0 with leaf curling and chlorosis; **C**, Estland (sensitive parent); **D**, F_2 plant of Estland × Col-0 without symptoms; **E**, F_2 plant with an intermediate response; **F**, sensitive F_2 plant.

between the tolerant lines, Col-0 or Col-0 *gl1*, and the sensitive lines, H55 and Estland (Table 2). F_1 plants from all of the crosses developed symptoms intermediate to those of the two parental lines (Fig. 2). Even after inoculation with TRSV, all of the F_1 plants survived and produced seeds but at reduced levels. Based on these results, it is likely that tolerance is controlled by the nuclear genome. The proportion of tolerant, intermediate, and sensitive progeny observed in F_2 plants was consistent with a 1:2:1 segregation ratio, indicating that the tolerance trait is controlled by a single locus in these crosses and that the tolerance gene is incompletely dominant with respect to the sensitivity gene (Table 2, Fig. 2). When F_3 families derived from 25 tolerant and 16 sensitive individuals were inoculated with TRSV the expression of tolerant phenotypes did not segregate further in tolerant and sensitive F_3 families (data not shown), further suggesting that the tolerance trait is controlled by a single locus. The tolerance locus that segregates in the progeny of crosses between Col-0 and Estland was named *TTR1*, for "tolerance to tobacco ringspot." F_1 plants of Estland × Col-0 and Col-0 *gl1* × H55 had different symptoms to TRSV infection. TRSV-infected F_1 plants of Estland × Col-0 were chlorotic and stunted, while F_2 plants of Col-0 *gl1* × H55 only had leaf curling. To determine if the sensitive genes in lines H55 and Estland are in the same complementation group, reciprocal crosses between lines H55 and Estland were made. All infected F_1 and F_2 plants of reciprocal crosses died within 10 days of inoculation with TRSV (data not shown), suggesting that both of the lines are sensitive due to the same locus. The possibility remains, however, that sensitivity in the F_1 is due to concurrent heterozygosity at two separate incompletely dominant tolerance loci.

Seed transmission of TRSV in *Arabidopsis*.

The rate at which TRSV infection was transmitted through seed in *Arabidopsis* was over 80% for most lines tested with the exception of Ler-0 which had a seed transmission rate of 60% (Table 3). The progeny from infected Col-0 were symptomless but over 97% were infected by TRSV, as detected by ELISA (Table 3). Because of the high seed transmission rate from F_1 seeds, the segregation of *TTR1* in F_2 seedlings could be evaluated without further mechanical inoculations. The 1:2:1 segregation ratio of 1 symptomless:2 leaf curling:1 seedling death observed in inoculated F_2 plants was also observed in the progeny from infected F_1 plants of crosses of Col-0 *gl* × H55 and Estland × Col-0. These data from F_2 plants infected by seed transmission further support the conclusion drawn from inoculated F_2 seedlings that the tolerance trait is controlled by a single locus. Most of the symptomless F_2 plants from these infected F_1 plants were infected by TRSV, as detected by ELISA. The F_3 progeny from infected tolerant F_2 plants were all tolerant and most were infected (Table 3). Infected F_2 plants displaying intermediate disease reaction produced F_3 progeny displaying essentially the same 1:2:1 segregation ratio and range of phenotypes as the original F_2 population (Table 3). This again suggests a single gene for tolerance in these populations.

Table 3. Seed transmission rate of TRSV in some *Arabidopsis* lines and segregation of *TTR1* in progeny of infected F_1 and F_2 plants of Col-0 *gl* × H55 and Estland × Col-0

Parental genotype	Progeny				
	Tolerant	Leaf curling	Seedling death	Not infected	Seed transmission (%)
Col-0	84	0	0	4	95
Ler-0	19	0	0	12	60
Ws-1	72	0	0	4	95
Col-4	33	0	0	3	92
Col-0 *gl*	64	0	0	0	100
F_1 of Est. × Col-0	12	18	10	7	85
F_1 of Col-0 *gl* × H55	39	75	30	6	96
F_2 of Est. × Col-0[a]	12	27	14	11	83
F_2 of Col-0 *gl* × H55[b]	62	0	0	1	98
F_3 of Col-0 *gl* × H55[b]	33	0	0	2	94
F_3 of Col-0 *gl* × H55[a]	50	57	43	8	96

[a] F_2 parent of these plants displayed intermediate symptoms.
[b] F_2 parent of these plants displayed tolerant symptoms.

Chromosomal location of the tolerance locus *TTR1*.

A set of genetic markers widely dispersed throughout the *Arabidopsis* genome was chosen for linkage analysis. Seventeen markers displayed polymorphism between Col-0 and

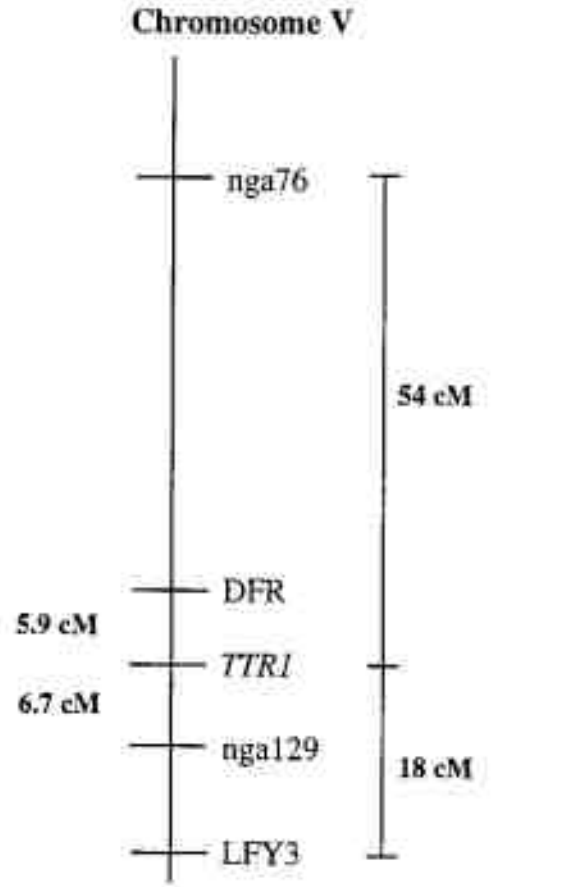

Fig. 3. The tolerance locus, *TTR1*, was positioned/mapped between nga129 and DFR markers on chromosome V. The figures indicate genetic distance between these markers and the target locus; cM = centimorgans.

Estland, including 11 SSLP markers (nga6, nga8, nga32, nga59, nga76, nga106, nga111, nga128, nga168, nga225, and nga248); five CAPS markers (LFY3, DHS1, m246, AG, and GAPA) and one visible marker (*gl1*). Initial mapping using 31 F_2 families indicated that the tolerance phenotype was linked only to markers LFY3 and nga76, which are both located on chromosome V. Recombination frequencies were 15% for LFY3 and 27% for nga76. Further mapping was conducted using 42 additional F_2 families and the markers nga129 and DFR, which are located between nga76 and LFY3. The tolerance locus *TTR1* was linked within 6.7 cM of nga129 and 5.9 cM of DFR on chromosome V. Three-point data indicated that the *TTR1* is located between the markers DFR and nga129 (Fig. 3, data not shown).

DISCUSSION

Several systems have been established to study the resistance of *Arabidopsis* plants to virus infection (Melcher 1989; Ishikawa et al. 1991; Simon et al. 1992; Leisner et al. 1993; Lee et al. 1994; Takahashi et al. 1994). We have developed an experimental system for the study of plant tolerance to virus infection. In our study, line Col-0 and many other *Arabidopsis* lines were found to be tolerant to TRSV infection, while nine lines including H55 and Estland were sensitive. Usually, Col-0 were symptomless after inoculation with TRSV although occasionally a few plants were stunted when inoculated with very high concentrations of virus. However, unlike the sensitive plants, these Col-0 individuals recovered and developed into normal-appearing plants. By a standard inoculation method, no visible symptoms were observed on infected Col-0 or Col-0 *gl1*. TRSV as measured by coat protein accumulated in Col-0 and sensitive Estland essentially to the same level and at the same rate. It appears that Col-0 does not restrict virus invasion, replication, or local or long distance movement, including movement into seed. Tolerance in Col-0 (and probably many other *Arabidopsis* lines) is apparently due to an absence of symptom development as the growth of infected plants was not adversely affected. This could be due to active suppression of symptom development, to passive insensitivity to virus replication, or to active response by sensitive lines. For example, Col-0 may constitutively express a functional tolerance gene whose expression leads to a reduction in symptoms in response to TRSV infection, or may be able to recognize TRSV viral proteins and induce a tolerance response which does not result in an HR. Alternatively, Col-0 and other tolerant lines may lack a functional protein that, in sensitive lines, allows interaction with the virus that leads to sensitivity.

TRSV has a very broad host range. Many infected host plants are asymptomatic (Stace-Smith 1985). Tolerance may represent a common host response to TRSV infection. *Arabidopsis* apparently did not evolve a specific host-pathogen interaction with TRSV that would lead to a specific gene-for-gene interaction. When Col-0, H55, and Estland were inoculated with two additional TRSV strains, symptoms on these three lines were similar to those caused by the TRSV-grape strain. All except nine of the 97 *Arabidopsis* lines displayed tolerance to TRSV infection. These results support the idea that tolerance to TRSV infection is a typical response of *Arabidopsis*. However, HRs have been observed in *Arabidopsis* in response to several plant viruses (Leisner and Howell 1992; Simon et al. 1992; Lee et al. 1994; Takahashi et al. 1994). From the results of crosses of Estland and H55, it appears that both sensitive lines have the same gene for tolerance. The different responses of Estland and H55 to TRSV infection may be due to different alleles of *TTR1* or to other physiological differences between these two lines. We found that the time during which *Arabidopsis* seedlings are susceptible to infection by TRSV is relatively narrow. The plants are very susceptible at the four- to five-leaf stage under high light intensity. Fewer plants became infected if inoculated after the six-leaf stage. This is very similar to the "developmental resistance" described in *Arabidopsis* for infection to cauliflower mosaic virus (Leisner et al. 1993).

General mechanisms of resistance and tolerance are apparently conserved among diverse plant species. This concept is supported by reports that suggest the existence of similar disease response genes in *Arabidopsis* and other crop plants (Whalen et al. 1988; Staskawicz et al. 1995). In soybeans, no effective sources of TRSV resistance or tolerance have been identified. In initial studies, we screened more than 700 soybean germplasm lines using the grape and bud blight strains of TRSV (J.-M. Lee and G. L. Hartman, unpublished). Most infected soybean plants, unlike most *Arabidopsis* lines, were sensitive and developed bud blight symptoms, possibly because of the narrow genetic base of soybeans compared to that of *Arabidopsis*. Use of *Arabidopsis* should facilitate study of tolerance as well as the isolation and characterization of TRSV tolerance gene(s) from *Arabidopsis* or from soybean and other agronomic crops.

Because of seed transmission of TRSV in *Arabidopsis*, fairly high rates of infection can be achieved by using the progeny of TRSV-infected plants. We used this method to confirm that the tolerant trait was controlled by a single locus and to screen a large number of the F_2 individual plants generated from infected F_1 plants. With this method, without further mechanical inoculations, we were able to screen thousands of F_2 seeds from infected F_1 plants to identify homozygous F_3 families or F_2 individuals. As a further test of this method, we are using seed transmission to screen for transposon insertion events into *TTR1* in *Arabidopsis* Ds/Ac lines. Virus-infected plants heterozygous for *TTR1* function due to insertion of a Ds or Ac element at the targeted locus are expected to display chlorosis and stunting. In addition, fine mapping is in progress to more precisely locate the tolerance locus by testing more markers on a large number of F_2 individuals identified by seed transmission. By studying the genetic and molecular basis of the sensitivity and tolerance responses, we hope to gain a better understanding of the molecular basis of the interaction of viral gene products with host factors and the ways in which this interaction contributes to viral disease.

MATERIALS AND METHODS

TRSV strains.

Three strains of tobacco ringspot virus have been used in this study. TRSV grape strain was obtained from ATCC (ATCC pv157). TRSV bud blight strain was obtained from G. Bruening, University of California, Davis, CA. One field collection of TRSV was isolated from soybean germplasm as seedborne virus in Urbana, IL.

Arabidopsis lines and plant growth.

Ninety-six lines obtained from Nottingham Seed Center and Ohio State University Genetic Center were used for initial screening. Col-0 and Col-0 *gl1* were our laboratory lines (originally obtained from S. Somerville and C. Somerville at Michigan State University). Single seeds were planted in 5-cm-diameter peat pots. Seeds were vernalized for 2 days at 4°C and then moved to 22°C with a day length of 16 h under 100-180 $\mu E\ m^{-2}\ s^{-1}$ light intensity. Plants were subirrigated weekly with nutrient medium.

Virus purification and inoculation.

TRSV bud blight and grape strains were purified from soybean (*Glycine max* (L.) Merr. cv. Williams) (Steere 1956). Soybean seedlings were inoculated at the two leaf stage. Trifoliolate leaves were collected and TRSV was purified. *Arabidopsis* plants having four to five fully developed leaves were mechanically inoculated with 2.3 μg/ml of TRSV in phosphate-buffered saline (PBS), pH 7.4 with Carborundum (600 mesh) or were mock-inoculated with PBS buffer. Plants were grown under the conditions described above. The 97 *Arabidopsis* lines were inoculated with TRSV grape strain; Col-0, Col-0 *gl1*, Estland, and H55 were inoculated with all three stains; and the genetics and mapping studies were done using TRSV grape strain.

Detection of virus coat protein in TRSV infected plants.

The accumulation of viral coat protein was analyzed by ELISA. Each line was planted in two replicates with 16 plants in each block. Two plants were taken from each replicate each line per day after inoculation. Noninoculated upper leaves of each plant were collected and weighed. Leaves were ground in 1 ml of PBS buffer per gram leaf tissue. Samples (100 μl) were taken for ELISA analysis. TRSV antibody and TRSV-conjugated alkaline phosphatase were purchased from Agdia, Inc. (Elkhart, IN). Alkaline phosphatase substrate kit was obtained from Bio-Rad Laboratories (Richmond, CA). ELISA was carried out according to the manufacturer's recommendation. Plates were read at 405 nm by EL340 reader (Bio-Tek Instruments, Winooski, VT).

Genetic analysis.

To analyze the genetics of the response to TRSV, reciprocal crosses were made between the sensitive line Estland and the tolerant line Col-0, and between the tolerant isoline Col-0 *gl1* and the sensitive line H55. F_1 and F_2 seeds were collected and plants were tested by TRSV as described above. Similarly, reciprocal crosses between H55 and Estland were also made. Crosses were performed according to the method described by Guzman and Ecker (1990). When scoring F_3 families for tolerance phenotype, a minimum of 12 F_3 individuals were tested.

Seed transmisssion of TRSV in _Arabidopsis_.

Seed transmission rate of TRSV in *Arabidopsis* was determined by inoculating line Col-0 and Col-0 *gl* and the F_1 and F_2 plants of crosses of lines Col-0 *gl* × H55 and Est. × Col-0 and collecting seeds from the infected plants. Infection of TRSV in the progeny was detected by visual observation and by ELISA.

Selection of the F_3 families and F_2 individuals for mapping.

Two methods were used to identify Col-0 × Estland F_2 families homozygous for the tolerance locus. First, healthy F_2 families were collected and then mechanically inoculated to test the responses of the families to TRSV. Sensitive and tolerant families were selected by this method for mapping. The second method was based on seed transmission of TRSV in *Arabidopsis*. The F_1 plants of lines Estland × Col-0 were inoculated with TRSV. The F_2 seeds from the infected F_1 plants were collected and planted. The symptomless F_2 plants were tested by ELISA. Infected tolerant F_2 plants were identified and the F_3 seeds from the tolerant F_2 plants were collected. Only tolerant F_3 families were selected by this method.

Chromosomal location of the tolerance locus.

Thirteen SSLP markers, nga6, nga8, nga32, nga59, nga76, nga106, nga111, nga128, nga168, nga225, nga248 (Bell and Ecker 1994); seven CAPS markers, LFY3, DHS1, m246, AG, GAPA (Konieczny and Ausubel 1993); and one visible marker *gl1* were tested on Col-0 and Estland to identify polymorphisms. All of the primers of the SSLP and CAPS markers were purchased from Research Genetics, Inc. (Huntsville, AL). For most markers, the PCR conditions were followed as published previously by Bell and Ecker (1994) for SSLP markers and Konieczny and Ausubel (1993) for CAPS markers. The markers that displayed polymorphisms between Col-0 and Estland were initially tested on a minimum of 31 F_3 families homozygous for tolerance or senstitivity. Chi-square tests were used to test for absence of linkage between molecular markers and the tolerance phenotype. Upon identification of linkage for markers LFY3 and nga76, the markers nga129 and DFR were used to more precisely define the map position of *TTR1* using 42 additional tolerant and sensitive F_2 families. Map distance were calculated using the formula $RF = \frac{1}{2}(1 - e^{-m})$, where RF = recombinant frequency and m = mean number of crossovers per meiosis or genetic map unit (Griffiths et al. 1996).

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