Field Evaluation of *Tomato spotted wilt virus* Resistance in Transgenic Peanut (*Arachis hypogaea*)

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ABSTRACT

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Spotted wilt, caused by *Tomato spotted wilt virus* (TSWV), is a devastating disease of many crops including peanut (*Arachis hypogaea*). Because the virus has a broad host range and is spread by ubiquitous thrips, disease management by traditional means is difficult. Developing new peanut cultivars with resistance to TSWV presents a significant challenge since existing genetic resistance in peanut germ plasm is limited. A genetic engineering approach appears to have great potential for resistance enhancement to TSWV. Transgenic peanut progenies that expressed the nucleocapsid protein of TSWV were subjected to natural infection of the virus under field conditions during the growing seasons of 1999 and 2000 in Tifton, GA, and in three locations (Tifton, GA, Marianna, FL, and Headland, AL) in 2001. Significantly lower incidence of spotted wilt was observed for the transgenic progeny in comparison to the nontransgenic checks in the field (in multiple years and locations) as well as during challenge inoculation under controlled environmental conditions. This transgenic event could potentially be used in a traditional breeding program to enhance host resistance.

Additional keywords: *Bunyaviridae*, groundnut, pathogen-derived resistance, plant transformation, *Tospovirus*

Peanut crop losses to *Tomato spotted wilt virus* (TSWV), the causal agent of spotted wilt disease, have reached economically significant levels (as much as an annual reduction of 12% in crop value in Georgia, which translated into more than $40 million [8,43]). A decline in losses after 1997 was due to adoption by growers of a combination of moderate host-plant resistance and changes in cultural practices (8). The virus is vectored by thrips (tobacco thrips, *Frankliniella fusca* Hinds, and western flower thrips, *Frankliniella occidentalis* Pergande) in Georgia; however, no single method such as insecticide application can significantly reduce spotted wilt in peanut, and integrated management systems are essential to minimize crop losses (11).

TSWV, a member of the genus *Tospovirus*, has a tripartite genome composed of single-stranded RNAs designated L, M, and S (1,30). The L RNA is of negative polarity and codes for the RNA-dependent RNA polymerase of ~330 kDa. The M and S RNAs are both in an ambisense arrangement. The M RNA contains two open reading frames, one that codes for a nonstructural protein (NSm) of 33.6 kDa, which is a putative viral cell-to-cell movement protein (24), and the second that codes for the precursor of the membrane glycoproteins G1 (78 kDa) and G2 (58 kDa) (23). The S RNA is approximately 2.9 kb with two open reading frames that encode a 52-kDa nonstructural protein (NSs) and a 29-kDa nucleocapsid protein (NP). The genomic RNAs are tightly bound to the viral NP forming a ribonucleoprotein complex, which is surrounded by a glycolipid membrane.

Among the five viral genes, the gene coding for the NP has been widely used to impart pathogen-derived virus resistance. Transgenic resistance to TSWV was first achieved by introducing the TSWV NP gene into *Nicotiana tabacum* plants (17,26). Since then, the generation of virus resistance through transgenic expression of the viral sequences has been reported in *N. benthamiana* (31,42), lettuce (32), tomato (19,22,40), *Chrysanthemum* (38), and *Osteospermum ecklonis* (41). Genetically engineered resistance in peanut has also been actively investigated in recent years (25,27,44), although results from only a single field trial with a small number of plants have been published (27). Previously, we reported the recovery of transgenic peanut plants via microprojectile bombardment that contained the NP gene of the lettuce isolate of TSWV (44). Southern blot analysis of independent transgenic lines confirmed the integration of one to several copies of the NP transgene into the peanut genome. Northern and enzyme-linked immunosorbent assay (ELISA) analyses suggested that a gene silencing mechanism may be operating in the primary transgenic lines containing multiple insertions of the NP transgene. One transgenic line that contained a single copy of the transgene showed expression of both mRNA and the NP in the primary transformant and the progeny. In this paper, we report the results of 3 years of field trials, as well as challenge inoculation under controlled conditions, on the response of an advanced progeny from the NP-expressing transgenic line to TSWV infection.

MATERIALS AND METHODS

Plant materials. Transgenic event 62-2a was selected from several transgenic plants that had been recovered after microprojectile bombardment of embryogenic tissues of cultivar Marc I (*Arachis hypogaea* L.) (18). The transgene consisted of the NP gene of the BL isolate of TSWV under the control of the *Cauliflower mosaic virus* (CaMV) 35S promoter (44). Event 62-2a had been shown to contain a single copy of the transgene, to express the transgene at the RNA and protein levels, and to transmit the transgene in Mendelian fashion (44).

Field test in 1999. Field tests were conducted at the Tifton Campus of the University of Georgia. Planting of 474 R_3 seeds (from 95 R_2 seedling progenies of five ELISA-positive R_1 lines) from transgenic

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*The *e*-Xtra logo stands for "electronic extra" and indicates that Figure 1 appears in color online.

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event 62-2a took place on 16 June 1999. Nontransgenic cultivars Georgia Runner and Tamrun 88 (5) were used as susceptible checks since Marc I is intermediate in its field reaction to TSWV compared with Georgia Green and Tamrun 88 (10,13). Checks were planted in two border rows surrounding the six rows of transgenic plants. Seeds were planted 10 to 15 cm apart. This plot was primarily intended to be used as a seed increase; therefore, sublines were planted in numerical sequence since replication was not possible. Transgenic plants, nontransgenic segregants, and nontransgenic checks were exposed to natural infection for more than 4 months beginning from the time of germination in late June until the end of the growing season. Plots received no insecticides for thrips control. Viral infection was visually evaluated and the numbers of plants showing symptoms of TSWV were recorded. To monitor disease progress, infection was rated on 30 July, 30 August, and 30 September. Symptoms included concentric ringspots, chlorosis, and bronzing of fully expanded leaves. On the terminal bud, symptoms could be seen as stunting, distortion, and necrosis of younger leaves. Plants with symptoms on at least one leaflet were counted as symptomatic. Although asymptomatic infection of peanut with TSWV is possible (12), we did not test for its occurrence.

Field test in 2000. A field test was conducted at the Tifton Campus of the University of Georgia (Lang Farm, Tift Co., GA) in a Tifton sandy loam (pH 5.8). The planting date was 9 June 2000. TSWV and both vector species occurred naturally at this location. Plots of lines of transformed Marc I peanut were compared to TSWV-susceptible Marc I and the moderately resistant cultivar Georgia Green (6). A completely randomized experimental design was used.

The test consisted of 60 rows of various sublines from transgenic event 62-2a, plus 10 rows each of Georgia Green and Marc I. Each plot was two rows of 6.1-m length and 0.9 m apart, and plots were separated length-wise by 2.4-m fallow alleys. Each row was planted with a different entry. Seeding rate was 12.3 seeds per meter of row for all entries, which was lower than the 19.2 plants per meter recommended for commercial production (4). Lower plant densities also promote a higher incidence and severity of spotted wilt disease (7). Plots received no insecticides for thrips control, but otherwise were maintained as recommended for peanut production and management of fungal diseases.

The extent of TSWV infection was evaluated at the end of October using a disease intensity rating that represented a combination of incidence and severity, as described by Culbreath et al. (16). The number of 0.31-m portions of row containing severely stunted, chlorotic, wilted, or dead plants was counted for each plot, and those numbers were converted to percentages of total row length for comparison of the lines. In addition, viral infection for each of 400 plants (that had been randomly selected and screened immediately after germination for expression of NP using ELISA) was visually scored on 18 September and 12 October. Plots were dug and inverted on 8 November and were allowed to dry in the windrow for 5 days. After that time, they were harvested mechanically with a stationary peanut thresher.

Field test in 2001. Field tests were conducted at the Tifton Campus of the University of Georgia (Lang Farm, Tift Co., GA), at the University of Florida, North Florida Research and Education Center (Marianna, Jackson Co., FL), and at the Auburn University Wiregrass Experiment Station (Headland, Henry Co., AL). The soil types were Tifton sandy loam (pH 5.8) at Tifton and Orangeburg loamy sand (pH 6.0) at Marianna and Headland. Randomized complete block designs with six replications (except for 00-18, which only had four replications) were used in all tests. Treatments consisted of seven genotypes, including five sublines derived from a single event of Marc I (line 62-2a) transformed with the NP gene from TSWV. The treatments also included Marc I and Georgia Green cultivars as checks. Both vector species and TSWV occurred naturally at each location.

Planting dates were 26 April 2001 in Tifton, 25 April 2001 in Marianna, and 24 April 2001 in Headland. In the southeastern United States, planting in April or June is typically more conducive for severe spotted wilt epidemics than planting in May (7). Seeding rate and plot size were as described for the 2000 field test except that each entry was planted in both rows of a plot. Each plot was bordered on one side by SunOleic 97R, a highly susceptible cultivar (15), but was adjacent on the other side to another randomly assigned plot. Plants in each plot were counted 14 to 21 days after planting (DAP) in each year to determine initial plant populations to ensure that differences observed in spotted wilt ratings were not due to differences in plant density. Spotted wilt intensity was evaluated in each plot, in the same manner as the previous year, on 19 June, 11 July, and 24 July at Tifton; 27 June, 26 July, and 16 August at Marianna; and 27 June, 26 July, and 16 August at Headland.

All tests were maintained as recommended for commercial production. Chlorothalonil (Bravo WeatherStik 720 F) or tebuconazole (Folicur 3.6 F) was applied as foliar sprays at 7- to 14-day intervals for control of foliar and/or soilborne fungal diseases. Plants of all entries, except Georgia Green, were dug and inverted 140 DAP at Tifton, 128 DAP at Marianna, and 119 DAP at Headland. Georgia Green, a later maturing variety, was dug 140 DAP at Tifton, 138 DAP at Marianna, and 133 DAP at Headland. Inverted plants were dried in windrows for 4 to 6 days. Pods were harvested mechanically, and pod yields were determined for each plot.

Serological assay of field-grown plants by ELISA. To check for expression of the NP gene of TSWV, double-antibody sandwich (DAS)-ELISA was conducted on leaf samples of transgenic plants using an ELISA kit (Agdia, Elkhart, IN) according to the manufacturer's protocol. In 1999, all 432 plants were tested, and in 2000, 400 samples out of 48,000 plants were selected for testing. Young leaf tissues (100 mg) were collected just after germination. Samples were prepared by grinding leaf tissues in 1 ml of an extraction buffer (phosphate-buffered saline, 0.05% Tween 20, 2% polyvinylpyrrolidone 40, 0.2% powdered milk, and 3 mM NaN_3). Reactions were allowed to proceed for 2 h then terminated by addition of 50 µl of 3 M sulfuric acid. Absorbance values were measured at 490 nm with an automated microplate reader (ELX 808; Bio-TEK Instruments, Winooski, VT).

Mechanical inoculation. Transgenic and nontransgenic lines of peanut were evaluated for their response to TSWV by mechanical inoculation. Georgia Runner and C11-2-39 were included as susceptible and resistant standards, respectively (29). A TSWV isolate collected from naturally infected lettuce in southeastern Georgia that produced a large number of local lesions and severe systemic necrosis in *Nicotiana tabacum* cv. K326 was used for inoculations. Mechanical inoculations were done as described by Mandal et al. (28). Inoculum was prepared by grinding infected tissues at the rate of 1:6 (wt/vol) tissue to buffer ratio in freshly prepared ice-cold 0.1 M potassium phosphate buffer, pH 7.0, containing 0.2% sodium sulfite and 0.01 M mercaptoethanol in a chilled mortar and pestle. Debris was removed by squeezing the extract through a pad of nonabsorbent cotton. Celite 545 and Carborundum 320-grit (Fisher Scientific, Fair Lawn, NJ) were added to the inoculum at final concentrations of 1 and 2%, respectively. The inoculum was maintained on ice for a maximum of 20 min until the inoculation was completed. Peanut plants, grown in pots, at two- to three-leaf stages (7 to 8 days after sowing) were dusted with Carborundum, and inoculum was applied only by rubbing the surfaces of the leaf with a Johnson's cotton swab (Johnson & Johnson, Skillman, NJ). After inoculation, the plants were sprayed with distilled water and kept in the growth chamber at daily temperatures ranging between 25 to 30°C with alternating light and dark periods of 12 h each (intensity of 15 to 20 klx) and 60 to 95% relative humidity. Inoculated plants were tested by ELISA 15 days postinoculation to confirm TSWV infection. Since the transgenic plants were expressing the NP

gene, TSWV infection was determined by using a monoclonal antibody specific to the NSs protein of TSWV (2).

Statistical analysis. Data were subjected to analysis of variance or chi-square goodness-of-fit tests using SAS software (Release 7.00, SAS Institute, Inc., Cary, NC). In 2001, data were analyzed across locations to check for location × genotype interactions. Fisher's protected least significant difference (LSD) values were calculated for comparison of genotypes. Differences described in the text are significant at $P \leq 0.05$ unless otherwise indicated.

RESULTS

Field test in 1999. Disease incidence in transgenic plants was significantly lower than in the susceptible checks on all three evaluation dates (Table 1). Incidence of infection for the transgenic plants ranged from 5 to 13%, whereas 12 to 49% of the checks were infected. The DAS-ELISA performed on a young leaflet shortly after germination showed that 329 out of 432 plants tested from the transgenic lines were positive for NP expression. Although the 432 plants were from 95 progenies, they represented the same event because they were originally derived from the same parent that had previously been shown to carry the NP gene at a single locus (44). Since the ELISA-negative segregants lacked NP, they could be used as an internal control for assessing the virus resistance level of the ELISA-positive plants. As a subset of the test, the ELISA-positive plants had a significantly lower level of infection than their ELISA-negative counterparts on all three screening dates (Table 2).

Field test in 2000. The DAS-ELISA showed that out of 400 randomly selected plants tested, 37 were negative and 363 were positive for NP expression. The increase in the percentage of ELISA-positive individuals in 2000 was likely due to our intentional selection of progeny from ELISA-positive parents in the 1999 field test. Viral infection of the 400 plants showed a significant difference on both rating dates between the ELISA-positive and ELISA-negative groups (Table 3). When the transgenic plots were compared with the check cultivars, the final spotted wilt intensity ratings were lower in the moderately resistant cultivar, Georgia Green, and in the transgenic lines than in the nontransgenic, susceptible cultivar, Marc I (Table 4). There also was a significantly greater yield for transgenic plants compared with the nontransgenic Marc I check (Table 4). The yields of transgenic plants and Georgia Green were not significantly different.

Field test in 2001. Transgenic sublines 00-11, 00-13, 00-18, and 00-51 were nearly homozygous for the NP gene since only 3% of the plants tested by ELISA shortly after germination for expression of NP were negative. Conversely, line 00-1/60 contained mostly (94%) nonexpressing plants and likely represented a nontransgenic segregant. The NP gene was not detected in nine nonexpressing plants, but was detected in one expressor, when assayed by the polymerase chain reaction (data not shown). This line had been planted as an additional control since it was derived from the transgenic line but no longer expressed NP.

Georgia Green and all experimental lines, except 00-1/60, had final spotted wilt intensity ratings that were lower than those of Marc I in all locations (Table 5). At Tifton and Headland, where epidemics were more severe, transgenic lines 00-11, 00-13, 00-18, and 00-51 had final intensity ratings that were lower than those of Georgia Green. Transgenic line 00-1/60, which actually contained very few ELISA-positive plants, was more similar in its disease severity to the Marc I check. The average percent total sound mature kernels ranged from 69 to 76 for Georgia Green, 66 to 71 for Marc I, and 69 to 77 for the transgenic Marc I lines, except for line 00-1/60, which was more similar to the Marc I check (65 to 72). Grades tended to be lower in Marianna and Headland than in Tifton.

Table 3. Incidence (symptoms) of spotted wilt disease in enzyme-linked immunosorbent assay (ELISA)-positive and -negative subsets of the transgenic line in 2000

ELISA	10 Sept. 2000 No. symptomatic plants/total (%)	χ^2	12 Oct. 2000 No. symptomatic plants/total (%)	χ^2
Positive	32/363 (9)		58/363 (16)	
Negative	11/37 (30)	15.3** [z]	16/37 (43)	16.6**

[z] ** significance level of 0.01.

Table 4. Rating of spotted wilt and yield for transgenic and check genotypes in the field in 2000 (Tifton, GA)

Genotype	Number of plots	Spotted wilt rating[y]		Yield (kg/plot)	
Transgenic Marc I	60	13.0 a	F = 65.4**[z]	2.90 a	F = 13.72**
Nontransgenic Marc I	10	55.8 b	LSD = 11.2*	1.89 b	LSD = 0.51*
Georgia Green	10	21.0 a		2.57 a	

[y] Percentage of linear row severely affected by spotted wilt disease; means within the column followed by different letters are significantly different.
[z] *,** significance levels of 0.05 and 0.001, respectively.

Table 1. Incidence (symptoms) of spotted wilt disease in peanut plants derived from a transgenic line and susceptible checks[y]

Genotype	30 Jul. 1999 No. symptomatic plants/total (%)	χ^2	30 Aug. 1999 No. symptomatic plants/total (%)	χ^2	30 Sept. 1999 No. symptomatic plants/total (%)	χ^2
Transgenic	23/432 (5)		46/432 (10)		58/432 (13)	
Georgia Runner	26/100 (26)	5.9* [z]	49/100 (49)	64.3**	49/100 (49)	57.8**
Tamrun 88	12/100 (12)	41.5**	44/100 (44)	81.4**	47/100 (47)	64.0**

[y] Chi-square tests compared each check with the transgenic line.
[z] *,** significance levels of 0.05 and 0.01, respectively.

Table 2. Incidence (symptoms) of spotted wilt disease in enzyme-linked immunosorbent assay (ELISA)-positive and -negative subsets of the transgenic line in 1999

ELISA	30 Jul. 1999 No. symptomatic plants/total (%)	χ^2	30 Aug. 1999 No. symptomatic plants/total (%)	χ^2	30 Sept. 1999 No. symptomatic plants/total (%)	χ^2
Positive	12/329 (4)		25/329 (8)		35/329 (11)	
Negative	11/103 (11)	7.7** [z]	21/103 (20)	13.5**	23/103 (22)	9.2**

[z] ** significance level of 0.01.

Mechanical inoculation. Inoculated transgenic and nontransgenic plants produced symptoms by 10 to 15 days postinoculation. The nontransgenic segregants and a susceptible standard, Georgia Runner, had similar levels of infection (93.3 and 91.9%), whereas only 48.9% of the inoculated transgenic plants became infected as judged by both symptoms and ELISA (Table 6). The resistant standard, C11-2-39, produced significantly more infected plants (77.1%) than the transgenic line (Table 6), but was not significantly different from the susceptible check, Georgia Runner. Throughout the study, symptoms in the transgenic line appeared milder compared with the nontransgenic line (Fig. 1).

DISCUSSION

The field ratings for incidence of spotted wilt indicated that transformation of a TSWV-susceptible runner-type peanut cultivar (Marc I) produced lines that exhibited significant levels of field resistance to TSWV. The relative performance of the various transformed lines followed similar trends across all locations in 2001, although a less severe epidemic in Marianna prevented a clear separation from checks. The level of resistance demonstrated by the near-homozygous transgenic lines was as good as or better than that of the moderately resistant cultivar Georgia Green. Georgia Green (6) has been shown to possess levels of resistance superior to Florunner (13), but the disease incidence can still rise above 40% (15; present study). Marc I previously has been reported to be similar to the susceptible cultivar Florunner with regard to field response to TSWV (10), and it has not been widely grown, perhaps partly because of its release (18) after TSWV had become a significant problem for peanut growers. Nevertheless, Marc I has other desirable characteristics such as flavor attributes (35) that make it a valuable member of germ plasm collections.

Using a highly efficient mechanical inoculation procedure, Mandal et al. (29) were able to distinguish the response of TSWV-resistant and susceptible genotypes under controlled conditions. Using the same approach, we found that the transgenic line had significantly fewer infected plants compared to the nontransgenic line as well as the resistant and susceptible standards. Compared to thrips-mediated natural infection, mechanical inoculation is likely to introduce a greater amount of virus inoculum, and the transgenic line had about half the number of infected plants compared to controls under identical growing conditions, indicating a relatively high level of resistance to TSWV.

For TSWV, the mechanism(s) that mediates pathogen-derived resistance is not fully resolved, although the possible mechanism(s) underlying transgenic resistance has been investigated by several groups (17,22,26,31–33). It appears that the resistance mechanism can operate either at the RNA level through post-transcriptional gene silencing (PTGS) (39) or at the protein level depending on the nature of the transgene and the insertion event (21,41). Resistance to multiple viruses was achieved by fusing sequences of NP genes from three tospoviruses in one transformation vector for tobacco (36). None of the transgenic plants expressed appreciable amounts of NP, but the resistance phenotypes varied from moderate (due to symptom delay), immune to one of the viruses but susceptible to the other two, to resistant to all three viruses. Clearly, the expression of transgene-dependent resistance is complex, although sequence-specific PTGS appears to be the most likely mechanism underlying the recovery of immunity in transgenic NP-gene containing lines. Substantial divergence between the infecting virus and the transgene sequences would be expected to overcome RNA-mediated resistance to TSWV even though this type of resistance has been shown to be more effective against the homologous isolate than protein-mediated resistance. Furthermore, PTGS can be reversed by some viruses, including TSWV, which encode suppressors that interfere with this RNA-based defense mechanism (3,9).

The mechanism of protein-mediated tospovirus resistance has not been determined, although Pang et al. (31) did show that replication of *Impatiens necrotic spot virus* could be inhibited in infected protoplasts from a TSWV-BL NP expressing line. Protein-mediated resistance often is manifested as a delay in or attenuation of symptom development, and the resistance may operate beyond a single serotype (17,31,42). Although we did not attempt to explore the mechanism of resistance in our materials, we predict that it is most likely to be protein mediated since a substantial amount of the NP is produced in our transgenic materials. The level of resistance observed in our transgenic lines is at least as high as that observed for the most widely grown resistant cultivar, Georgia Green.

Given that different mechanisms of resistance may be operating in different NP-gene containing lines, it is difficult to speculate on the potential durability of resistance. The NP gene of TSWV is rela-

Table 6. Response of transgenic peanut over three experiments following mechanical inoculation with *Tomato spotted wilt virus*[x]

	Number infected/inoculated			Average transmission
Genotypes	I[y]	II	III	%
Transgenic line	6/9	4/10	4/10	48.9 a[z]
Nontransgenic line	9/9	8/10	10/10	93.3 b
Resistant standard (C11-2-39)	6/10	5/7	10/10	77.1 b
Susceptible standard (Georgia Runner)	6/7	10/10	9/10	91.9 b
LSD ($P = 0.05$)				27.4

[x] Infection was judged by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody to NSs protein.
[y] Inoculation trials.
[z] Means with the same letter in the column are not significantly different at $P = 0.05$.

Table 5. Rating of spotted wilt and yield for transgenic and check genotypes in the field in Tifton, GA, Marianna, FL, and Headland, AL, in 2001

	Spotted wilt rating[z]			Yield (kg/plot)		
Genotype	Tifton	Marianna	Headland	Tifton	Marianna	Headland
Georgia Green	23.3	8.8	21.1	4.19	5.08	4.23
Marc I	58.3	15.8	41.9	2.77	4.79	2.10
Transgenic 00-11	12.1	3.8	4.8	5.80	5.17	3.94
Transgenic 00-13	6.7	2.9	3.5	5.80	5.67	3.72
Transgenic 00-18	16.3	5.6	9.1	4.78	4.83	3.36
Transgenic 00-51	9.4	5.0	5.2	5.00	5.14	3.30
Transgenic 00-1/60	60.6	26.5	50.6	2.14	4.42	2.06
LSD ($P = 0.05$)	6.7	6.5	11.0	0.80	0.40	1.01

[z] Percentage of linear row severely affected by *Tomato spotted wilt virus.*

tively conserved at the nucleotide level (>95%; 34); therefore, pathogen-derived resistance that is based on homology-dependent RNA silencing should remain stable in the absence of suppressors of silencing, although such suppressors already have been described in TSWV (9). It also has been shown that infection of tobacco by multiple isolates can result in reassortment of the subgenomic molecules, and this reassortment can be selected for in NP-gene transgenic lines (37). Although the reassortment did not cause a significant change in NP-gene sequence, it nevertheless resulted in a suppression of the resistance reaction. Perhaps the durability of resistance in environments with diverse virus populations could be enhanced by combining the NP-gene mediated resistance with natural host-plant resistance (20). Tolerance to *Groundnut ringspot virus* and one isolate of TSWV, conferred by the *Sw-5* gene in tomato, has been combined with tolerance to multiple TSWV isolates in transgenic lines containing the NP gene (20). Based on the results of our multiyear, multilocation field study to evaluate TSWV resistance in NP-gene expressing transgenic peanut, the potential exists to combine NP-mediated resistance in transgenic peanut with host-plant resistance that already has been identified in the peanut germ plasm. Currently, several peanut breeding programs are making significant progress in improving resistance to TSWV through traditional breeding methods (14,15). Stacked genes, likely conferring different resistance mechanisms, should provide more durable control of spotted wilt in peanut.

Fig. 1. Representative symptoms on peanut plants following mechanical inoculation by *Tomato spotted wilt virus*. Top, nontransgenic control; bottom, transgenic line.

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LITERATURE CITED

1. Adkins, S. 2000. Tomato spotted wilt virus – positive steps towards negative success. Mol. Plant Pathol. 1:151-157.
2. Bandla, M. D., Westcot, D. M., Chenault, K. D., Ullman, D. E., German, T. L., and Sherwood, J. L. 1994. Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of tomato spotted wilt tospovirus to identify viruliferous thrips. Phytopathology 84:1427-1431.
3. Baulcombe, D. 2002. Viral suppression of systemic silencing. Trends Microbiol. 10:306-308.
4. Beasley, J. P., Jr. 2003. Managing agronomic inputs: Improving profit potential with $355 per ton peanuts. In: 2003 Peanut Update. E. P. Prostko, ed. Univ. Georgia Coll. Agric. Environ. Sci. CSS-03-0105, Athens, GA.
5. Black, M. C., and Smith, D. H. 1987. Spotted wilt and rust reactions in south Texas among selected peanut genotypes. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 19:31.
6. Branch, W. D. 1996. Registration of 'Georgia Green' peanut. Crop Sci. 36:806.
7. Brown, S. L., Todd, J. W., and Culbreath, A. K. 1996. Effect of selected cultural practices on incidence of tomato spotted wilt virus and populations of thrips vectors in peanuts. Acta Hortic. 431:491-498.
8. Brown, S. L., Todd, J., Culbreath, A., Baldwin, J., Beasley, J., Kemerait, B., and Prostko, E. 2003. Minimizing spotted wilt of peanut. Univ. Georgia Coop. Ext. Serv. Bull. 1165.
9. Bucher, E., Sijen, T., De Haan, P., Goldbach, R., and Prins, M. 2003. Negative-strand tospoviruses and tenuiviruses carry a gene for a suppressor of gene silencing at analogous genomic positions. J. Virol. 77:1329-1336.
10. Culbreath, A. K., Todd, J. W., Branch, W. D., Brown, S. L., Demski, J. W., and Beasley, J. P., Jr. 1994. Effect of new peanut cultivar Georgia Browne on epidemics of spotted wilt. Plant Dis. 78:1185-1189.
11. Culbreath, A. K., Todd, J. W., and Brown, S. L. 2003. Epidemiology and management of tomato spotted wilt in peanut. Annu. Rev. Phytopathol. 41:53-75.
12. Culbreath, A. K., Todd, J. W., and Demski, J. W. 1992. Comparison of hidden and apparent spotted wilt epidemics in peanut. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 24:39.
13. Culbreath, A. K., Todd, J. W., Gorbet, D. W., Branch, W. D., Sprenkel, R. K., Shokes, F. M., and Demski, J. W. 1996. Disease progress of tomato spotted wilt virus in selected peanut cultivars and advanced breeding lines. Plant Dis. 80:70-73.
14. Culbreath, A. K., Todd, J. W., Gorbet, D. W., Brown, S. L., Baldwin, J. A., Pappu, H. R., Holbrook, C. C., and Shokes, F. M. 1999. Response of early, medium, and late maturing peanut breeding lines to field epidemics of tomato spotted wilt. Peanut Sci. 26:100-106.
15. Culbreath, A. K., Todd, J. W., Gorbet, D. W., Brown, S. L., Baldwin, J., Pappu, H. R., and Shokes, F. M. 2000. Reaction of peanut cultivars to spotted wilt. Peanut Sci. 27:35-39.

16. Culbreath, A. K., Todd, J. W., Gorbet, D. W., Shokes, F. M., and Pappu, H. R. 1997. Field response of new peanut cultivar UF 91108 to tomato spotted wilt virus. Plant Dis. 81:1410-1415.
17. Gielen, J. J. L., de Haan, P., Kool, A. J., Peters, D., van Grinsven, M. Q. J. M., and Goldbach, R. W. 1991. Engineered resistance to tomato spotted wilt virus, a negative-strand RNA virus. Bio/Technology 9:1363-1367.
18. Gorbet, D. W., Knauft, D. A., and Norden, A. J. 1992. Marc I peanut. Crop Sci. 32:279.
19. Gubba, A., Gonsalves, C., Stevens, M. K., Tricoli, D. M., and Gonsalves, D. 1998. Comparative tospovirus resistance of tomato with the Sv gene and transgenic tomato expressing the N gene of tomato spotted wilt tospovirus. (Abstr.) Phytopathology 88:S34.
20. Gubba, A., Gonsalves, C., Stevens, M. K., Tricoli, D. M., and Gonsalves, D. 2002. Combining transgenic and natural resistance to obtain broad resistance to tospovirus infection in tomato (*Lycopersicon esculentum* Mill). Mol. Breed. 9:13-23.
21. Herrero, S., Culbreath, A. K., Csinos, A. S., Pappu, H. R., Rufty, R. C., and Daub, M. E. 2000. Nucleocapsid gene-mediated transgenic resistance provides protection against *Tomato spotted wilt virus* epidemics in the field. Phytopathology 90:139-147.
22. Kim, J. W., Sun, S. S. M., and German, T. L. 1994. Disease resistance in tobacco and tomato plants transformed with the tomato spotted wilt virus nucleocapsid gene. Plant Dis. 78:615-621.
23. Kormelink, R., de Haan, P., Meurs, C., Peters, D., and Goldbach, R. 1992 The nucleotide sequence of the M RNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. J. Gen. Virol. 73:2795-2804.
24. Kormelink, R., Storms, M., van Lent, J., Peters, D., and Goldbach, R. 1994. Expression and subcellular localization of the NSm protein of tomato spotted wilt virus (TSWV), a putative movement protein. Virology 200:56-65.
25. Li, Z. J., Jarret, R. L., and Demski, J. W. 1997. Engineered resistance to tomato spotted wilt virus in transgenic peanut expressing the viral nucleocapsid gene. Transgen. Res. 6:297-305.
26. MacKenzie, D. J., and Ellis, P. J. 1992. Resistance to tomato spotted wilt virus infection in transgenic tobacco expressing the viral nucleocapsid gene. Mol. Plant-Microbe Interact. 5:34-40.
27. Magbanua, Z. V., Wilde, H. D., Roberts, J. K., Chowdhury, K., Abad, J., Moyer, J. W., Wetzstein, H. Y., and Parrott, W. A. 2000. Field resistance to tomato spotted wilt virus in transgenic peanut (*Arachis hypogaea* L.) expressing an antisense nucleocapsid gene sequence. Mol. Breed. 6:227-236.
28. Mandal, B., Pappu, H. R., and Culbreath, A. K. 2001. Factors affecting mechanical transmission of *Tomato spotted wilt virus* to peanut (*Arachis hypogaea*). Plant Dis. 85:1259-1263.
29. Mandal, B., Pappu, H. R., Culbreath, A. K., Holbrook, C. C., Gorbet, D. W., and Todd, J. W. 2002. Differential response of selected peanut (*Arachis hypogaea*) genotypes to mechanical inoculation by *Tomato spotted wilt virus*. Plant Dis. 86:939-944.
30. Mumford, R. A., Barker, I., and Wood, K. R. 1996. The biology of the tospoviruses. Ann. Appl. Biol. 128:159-183.
31. Pang, S.-Z., Bock, J. H., Gonsalves, C., Slightom, J. L., and Gonsalves, D. 1994. Resistance of transgenic *Nicotiana benthamiana* plants to tomato spotted wilt and impatiens necrotic spot tospoviruses: Evidence of involvement of the N protein and N gene RNA in resistance. Phytopathology 84:243-249.
32. Pang, S.-Z., Jan, F. J., Carney, K., Stout, J., Tricoli, D. M., Quemada, H. D., and Gonsalves, D. 1996. Post-transcriptional transgene silencing and consequent tospovirus resistance in transgenic lettuce are affected by transgene dosage and plant development. Plant J. 9:899-909.
33. Pang, S.-Z., Nagpala, P., Wang, M., Slightom, J. L., and Gonsalves, D. 1992. Resistance to heterologous isolates of tomato spotted wilt virus in transgenic tobacco expressing its nucleocapsid protein gene. Phytopathology 82:1223-1229.
34. Pappu, H. R., Pappu, S. S., Jain, R. K., Bertrand, P. F., Culbreath, A. K., McPherson, R., and Csinos, A. S. 1998. Sequence characteristics of natural populations of tomato spotted wilt tospovirus infecting flue-cured tobacco in Georgia. Virus Genes 17:167-175.
35. Pattee, H. E., Isleib, T. G., Gorbet, D. W., Giesbrecht, F. G., and Cui, Z. 2001. Parent selection in breeding for roasted peanut flavor quality. Peanut Sci. 28:51-58.
36. Prins, M., de Haan, P., Luyten, R., van Veller, M., van Grinsven, M. Q. J. M., and Goldbach, R. 1995. Broad resistance to tospoviruses in transgenic tobacco plants expressing three tospoviral nucleoprotein gene sequences. Mol. Plant-Microbe Interact. 8:85-91.
37. Qiu, W., and Moyer, J. W. 1999. Tomato spotted wilt tospovirus adapts to the TSWV N gene-derived resistance by genome reassortment. Phytopathology 89:575-582.
38. Sherman, J. M., Moyer, J. W., and Daub, M. E. 1998. Tomato spotted wilt virus resistance in chrysanthemum expressing the viral nucleocapsid gene. Plant Dis. 82:407-414.
39. Tijsterman, M., Ketting, R. F., and Plasterk, R. H. A. 2002. The genetics of RNA silencing. Annu. Rev. Genet. 36:489-519.
40. Ultzen, T., Gielen, J., Venema, F., Westerbroek, A., de Haan, P., Tan, M. L., Schram, A., van Grinsven, M., and Goldbach, R. 1995. Resistance to tomato spotted wilt virus in transgenic tomato hybrids. Euphytica 85:159-168.
41. Vaira, A. M., Berio, T., Accotto, G. P., Vecchiati, M., and Allavena, A. 2000. Evaluation of resistance in *Osteospermum ecklonis* (DC.) Norl. plants transgenic for the N protein gene of tomato spotted wilt virus. Plant Cell Rep. 19:983-988.
42. Vaira, A. M., Semeria, L., Crespi, S., Lisa, V., Allavena, A., and Accotto, G. P. 1995. Resistance to tospoviruses in *Nicotiana benthamiana* transformed with the N gene of tomato spotted wilt virus: Correlation between transgene expression and protection in primary transformants. Mol. Plant-Microbe Interact. 8:66-73.
43. Williams-Woodward, J. 2001. 2000 Georgia plant disease loss estimates. Univ. Georgia Coop. Ext. Serv. Pub. Path. 01-001, Athens, GA.
44. Yang, H. Y., Singsit, C., Wang, A., Gonsalves, D., and Ozias-Akins, P. 1998. Transgenic peanut plants containing a nucleocapsid protein gene of tomato spotted wilt virus show divergent levels of gene expression. Plant Cell Rep. 17:693-699.