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Transgenic peanut plants containing a nucleocapsid protein gene of tomato spotted wilt virus show divergent levels of gene expression

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Abstract The nucleocapsid protein (N) gene of the lettuce isolate of tomato spotted wilt virus (TSWV) was inserted into peanut (*Arachis hypogaea* L.) via microprojectile bombardment. Constructs containing the *hph* gene for resistance to the antibiotic hygromycin and the TSWV N gene were used for bombardment of peanut somatic embryos. High frequencies of transformation and regeneration of plants containing the N gene were obtained. Southern blot analysis of independent transgenic lines revealed that one to several copies of the N gene were integrated into the peanut genome. Northern blot, RT-PCR and ELISA analyses indicated that a gene silencing mechanism may be operating in primary transgenic lines containing multiple copy insertions of the N transgene. One transgenic plant which contained a single copy of the transgene expressed the N protein in the primary transformant, and the progeny segregated in a 3:1 ratio based upon ELISA determination.

Key words *Arachis hypogaea* · Genetic transformation · N gene · Somatic embryo · Tomato spotted wilt virus

Abbreviations *GUS* β-Glucuronidase · *hph* Hygromycin phosphotransferase gene · *N* Nucleocapsid · *MAR* Matrix attachment region · *TSWV* Tomato spotted wilt virus

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Introduction

Peanut is an important commercial crop worldwide. It provides an excellent source of protein and other nutrients. Unfortunately, peanuts suffer from many insect pests, fungal diseases, and numerous viral diseases. Tomato spotted wilt virus (TSWV), a member of the Tospoviruses, is one of the most serious and widespread pathogens of many economically important crop plants including peanuts (Mumford et al. 1996). In the US, TSWV has been responsible for a number of epidemics, including those in southern Texas where million dollar losses were incurred in 1985, 1986, and 1987 (Demski et al. 1990). In Georgia, TSWV has caused very heavy losses on tobacco, peanut, and pepper crops (Chamberlin et al. 1992).

TSWV has a wide host range of over 70 families of both monocotyledons and dicotyledons and is vectored by thrips (Thysanoptera: Thripidae) (Goldbach and Peters 1994), but it cannot be practically controlled by chemicals. Although variations in the disease tolerance among commercial peanut cultivars do exist (Branch 1996), a suitable resistant germplasm for traditional breeding is not available (Demski et al. 1990). Therefore, genetically engineered resistance has been actively investigated in recent years as an alternative (Li et al. 1997).

Coat-protein-mediated resistance, a form of pathogen-derived resistance, has been established as an effective means of protection against viral infection and the prevention of crop loss (Baulcombe 1996; Beachy 1997). Expression of viral capsid genes by transgenic host plants results in reduced susceptibility of the plant to virus infection (Powell-Abel et al. 1986; Grumet 1994) where the degree of protection ranges from a delay in symptom expression to absence of disease symptoms and virus accumulation. Coat protein genes have been shown to confer partial or complete resistance as was observed for tobacco streak virus in tobacco (Van Dun et al. 1988), cucumber mosaic virus in cucumber (Nishibayashi et al. 1996), and potato virus Y in potato plants (Hefferon et al. 1997). TSWV is an enveloped RNA virus with a tri-

partite genome and contains a nucleocapsid protein associated with each of the three RNA molecules. Three independent reports (Gielen et al. 1991; MacKenzie and Ellis 1992; Pang et al. 1992) demonstrated that transgenic plants expressing the nucleocapsid protein (N) gene of TSWV were resistant to infection by the isolate from which the N gene was cloned. However, variation in resistance did not closely correlate with the amount of N protein produced, and protection against isolates with high N gene homology to the transgene was largely RNA mediated (Mumford et al. 1996). Protein-mediated protection may play a role in the delay or attenuation of symptoms in plants infected with distantly related heterologous isolates (Pang et al. 1994). However, highly resistant transgenic lines displayed post-transcriptional transgene silencing resulting from RNA turnover (Pang et al. 1996, 1997). Since TSWV is widespread with many biologically diverse isolates, it is important to test the effectiveness of a single N gene for conferring resistance to different TSWV isolates and in different hosts. The objective of the present study was to insert the N gene into peanut somatic embryos via microprojectile bombardment and to assay for N protein production in transgenic plants.

Materials and methods

Plant materials and culture

Embryogenic cultures of peanut were initiated from immature cotyledons of three commercial cultivars, Florunner, Georgia Runner and MARC-1 as previously described (Ozias-Akins et al. 1993). Callus cultures were routinely transferred at 2-week intervals to Murashige and Skoog (1962) salts and vitamins, supplemented with 30 $g \cdot l^{-1}$ sucrose, 3 $mg \cdot l^{-1}$ picloram, 1 $g \cdot l^{-1}$ glutamine (filter-sterilized) and solidified with 8 $g \cdot l^{-1}$ agar. The pH was adjusted to 5.8 prior to autoclaving. All embryogenic cultures were maintained in the dark at 28 °C for up to 9 months and bombarded 2 weeks after subculture.

Plasmid constructs

The plant expression cassette containing the N gene coding sequence from the lettuce isolate of tomato spotted wilt virus (TSWV-BL) was obtained from Cornell University in a pBIN19 vector (Pang et al. 1992). The gene cassette (1.5 kb) was excised from pBIN19 by partial digestion with *Hin*dIII (the N gene coding sequence contains an internal *Hin*dIII site) and subcloned into another plant transformation vector, pCB13 (provided by David Ow, Plant Gene Expression Center, Albany, Calif.) at a unique *Hin*dIII site. The plasmid pCB13 contains the selectable marker gene hygromycin phosphotransferase (*hph*) conferring resistance to the antibiotic hygromycin. The resulting plasmid is referred to as pCB13-N^{+}.

A second plant expression cassette (CP-exp) containing the N gene was acquired from the Upjohn Company, Kalamazoo, Mich. The gene cassette contained a 400-bp promoter (fusion between a 330-bp CaMV 35 S and a 70-bp CMV 5′ untranslated region) and the CaMV 35 S terminator region, including the poly(A)-addition signal (Slightom 1991). The N gene cassette was excised from the plant expression vector CP-exp, and subcloned into the *Hin*dIII site of the pCB13 vector. The resulting plasmid is referred to as pCB13-N^{++}.

Microprojectile bombardment, selection, and regeneration

Six bombardment experiments (8–12 plates per bombardment; each plate contained 12–15 embryogenic pieces, total weight of approximately 1.5 g) were carried out using DNA from two plasmid constructs pCB13-N^{+} and pCB13-N^{++} and a PDS 1000/helium-driven biolistic device (Bio-Rad, Hercules, Calif.) as described previously (Ozias-Akins et al. 1993). The following modifications were added to the bombardment experiments with pCB13-N^{++}. Amounts of DNA delivered were increased from 1.0 to 1.66 μg per bombardment, selection was initiated in liquid medium 3–6 days post-bombardment instead of 7–14 days, and medium was changed weekly instead of at 2-week intervals. A three-step regeneration protocol was followed to obtain plants from stably transformed somatic embryos (Ozias-Akins et al. 1993).

PCR analysis of putative transformants

Putative transformants were screened first by PCR for the presence of *hph* and N genes. DNA was isolated from fresh embryogenic cultures or leaves according to Singsit et al. (1997) and amplification was carried out in a thermal cycler (Perkin-Elmer Cetus, Foster City, Calif.) under the following conditions: 94 °C for 30 s, 50 °C for 30 s, 72 °C for 2 min, for 35 cycles. The 384-bp region of *hph* was amplified using primer H830 (5′-GTT CAT TTC ATT TGG AGA G-3′) specific for the CaMV 35 S promoter and H706 (5′-GAA TTC CCC AAT GTC AAG CAC TTC CG-3′) specific to the *hph* coding region. A 950 bp region of the TSWV gene cassette was amplified using H705 (5′-ATA TCT CCA CTG ACG TAA GGG ATG ACG-3′) specific for the CaMV 35 S promoter and H240 (5′-TTA ACA CAC TAA GCA AGC AC-3′) specific to the N coding region.

Southern blot analyses

Genomic DNA (10 μg) was digested with *Hin*dIII and subsequently purified with Geneclean II (BIO 101, Vista, Calif.). After electrophoresis on a 0.8% agarose gel in 1×TBE, DNA was transferred to GeneScreen Plus nylon membrane (NEN Research Products, Boston, Mass.) using 0.4 N NaOH (Sambrook et al. 1989). Hybridization was carried out at 60 °C in a solution containing 7% SDS, 0.25 M NaH_2PO_4, 1 mM EDTA, and 1% bovine serum albumin (Church and Gilbert 1984). A DNA probe labeled with [α-^{32}P]dCTP was synthesized by PCR following the procedure of Schowalter and Sommer (1989). Two primers, TSWVS1 (5′-AAA ACT TCA GAC AGG ATT GGA GCC-3′) and TSWVA1 (5′-GCA GCA TAC TCT TTC CCC TTC TTC-3′), which amplified 410 bp of the N gene coding sequence, were used. Following hybridization, the membrane was washed for 5 min in 25 ml of 0.5×SSPE/1%SDS at room temperature, 30 min in 100 ml of 0.5×SSPE/1% SDS at 60 °C and 30 min in 100 ml 0.1×SSPE/1% SDS at 60 °C. After washing, the membrane was exposed to autoradiographic film at –80 °C.

Transgene expression by Northern, RT-PCR, and ELISA analyses

For the estimation of transgene expression by Northern blot analysis, total RNA was isolated from young leaves according to the procedure of Knapp and Chandle (1996). RNA was precipitated from the aqueous phase by the addition of a 1/2 vol of 7.5 M ammonium acetate, incubation on ice for 1 h, and centrifugation for 10 min at 14,000 *g*. The RNA pellet was washed twice in 3 M sodium acetate, pH 5.2, at room temperature (Logeman et al. 1987), washed once with 70% ethanol, dissolved in DEPC-treated dH_2O, and stored at –80 °C until use.

For electrophoresis, total RNA (10 μg) was electrophoretically fractionated through a 1.0% denaturing formaldehyde gel using 1×MOPS running buffer as described (Sambrook et al. 1989). RNA was transferred to GeneScreen Plus using 7.5 mM NaOH as transfer solution. Prehybridization and hybridization were carried out using the same procedure as described in the Southern analysis, except that the N-gene-specific radioactive probe was synthesized by random

Table 1 Number of hygromycin-resistant lines and lines testing positive for the genes by PCR (*hph* and N) or ELISA (N)

Experiment	Cultivar	Plasmid	Hyg^R line	PCR/hph	PCR/N	ELISA
T32/T33 (18 plates)	Florunner	N^+	10	9	6	3/3
T49 (12 plates)	GA Runner	N^+	6	6	6	5/6
T62 (12 plates)	MARC-1	N^{++}	25	25	25	7/11
T65 (12 plates)	Florunner	N^{++}	3	3	3	2/3
T68 (12 plates)	GA Runner	N^{++}	8	6	5	3/4
Total			52	49	45	20/27

priming (DECAPrime II Kit, Ambion, Austin, Tex.) of the *Nco*I fragment which spans the entire N gene cassette. To demonstrate equal loading, the blots were stripped in 70% formamide at 70 °C and rehybridized with a ribosomal RNA-specific probe generated using two sets of primers to the conserved regions of the gene. Part of the 18 s rDNA sequence (1 kb) was amplified from peanut using primers 18SF1 (5′-AAC GGC TAC CAC ATC CAA GGA AGG C-3′) and 18SR1 (5′-GCG CGT GCG GCC CAG AAC ATC TAA G-3′). The internal transcribed spacer region was amplified from peanut using ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and ITS5 (5′-GGA AGT AAA AGT CGT AAC A-3′) which lie in the conserved regions of the 18 s and 28 s genes of ribosomal DNA, respectively, and flank the ITS1, 5.8 s and ITS2 regions (White et al. 1990).

For the analysis of transgene expression using RT-PCR, mRNA isolated with the Dynabeads mRNA purification kit (Dynal, Great Neck, N.Y.) was used as a template. Young leaf tissues were ground to powder under liquid nitrogen by vigorous vortexing with ball bearings. Crude tissue extracts were centrifuged to remove cell debris before mixing with oligo(dT)25 Dynabeads to selectively bind poly$(A)^+$ RNA to the magnetic beads. The beads were then washed at least five times, each time with 1 ml washing buffer without SDS, and mRNAs were eluted in 60 µl of 2 mM EDTA (pH 7.5). For RT-PCR, a single-vial, two-stage protocol of Pappu et al. (1993) was followed in which reverse transcriptase and PCR reagents were combined in a single reaction mix containing 10 mM DTT, 100 µM of each dNTP, 20 pmol of each primer, 10 U of RNAsin (Promega), 50 mM KCl, 10 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 15 U of AMV Reverse Transcriptase (Promega), 5 U of Taq DNA Polymerase (Promega) and 10 µl of mRNA in a total volume of 100 µl. The mRNA templates were heated to 70 °C for 5 min and quickly chilled on ice before adding to the reaction mix. First-strand cDNA synthesis was achieved by incubation at 42 °C for 45 min using the antisense oligonucleotide primer H240. The reverse transcription was directly followed by PCR amplification using N-gene-specific primers (TSWVS1 and H240) under the following conditions: 2 cycles of 94 °C/2 min, 55 °C/1 min, and 72 °C/2 min; 38 cycles of 92 °C/10 s, 55 °C/7 s, and 72 °C/90 s, and soak at 6 °C. To monitor genomic DNA contamination, a control reaction for each sample was run with all reaction components except reverse transcriptase.

To detect expression of the N gene at the protein level in transgenic peanut plants, double-antibody sandwich enzyme-linked immunosorbant assay (DAS-ELISA) (Clark and Adams 1977) was carried out with a TSWV-N-specific antibody and 100 µl of sample extract using the ELISA kit from Agdia (Elkart, Ind.) according to the manufacturer's protocol. Young leaf tissues were ground in 10 vol of phosphate-buffered saline-Tween extraction buffer (0.14 M NaCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4, 0.05% Tween-20, and 3 mM KCl, pH 7.4) containing 10 mM Na_2SO_4, 2% (wt/vol) polyvinylpyrrolidone-40, 2% Tween-20, 0.2% (wt/vol) 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.).

Results and discussion

The results presented here demonstrate the incorporation of the N gene of the lettuce strain of the TSWV (Wang and Gonsalves 1990) into the peanut genome of three commercial peanut cultivars, Florunner, Georgia Runner, and MARC-l. Fifty-two hygromycin-resistant cell lines were derived from six bombardment experiments of which 49 cell lines were positive for the *hph* gene and 45 were positive for the N gene when assayed by PCR using two primers specific to the CaMV 35 S promoter region and two others specific for the *hph* and Ngene coding regions, respectively (Table 1). The three putative escapes were not analyzed with a second DNA preparation and could reflect false-negative results. Based on these data, a maximum of 6% escapes can be expected. The cotransformation frequency for the two covalently linked genes was greater than 90% in the cell lines. The total frequency of transformation appears to be somewhat greater in MARC-1 compared to Florunner and Georgia Runner, and we speculate that this may be due to the presence of less differentiated somatic embryos in MARC-1 cultures. Putative primary transgenic plants analyzed by PCR also showed that the majority were cotransformed with both the *hph* and N genes (Fig. 1). Expression of the N gene at the protein level was detected by ELISA in only 74% of the embryogenic lines analyzed. Undetectable protein could have reflected expression below the limit of sensitivity of the ELISA assay or complete lack of expression due to gene rearrangement or gene silencing, either transcriptional or post-transcriptional.

Many of the primary transgenic plants in the greenhouse had to be destroyed because of an inadvertent TSWV outbreak and clearly did not display resistance to the pathogen. Most of the plants from the T62 experiment survived and several were highly fertile; therefore, subsequent molecular analyses were carried out only on these lines. Southern blot analysis of genomic DNA from transgenic plants revealed that the N gene was integrated into the peanut genome of different individuals with diverse copy numbers and insertion sites (Fig. 2). The transgenic R_0 plants 62-2a3, 62-2a6, 62-2d1, and 62-2d2 showed only a single band of the same size as the *Hin*dIII fragment of the N gene. In the remaining transgenic R_0 plants analyzed there were numerous other hybridizing bands of higher molecular weights in addition to the strongly hybridizing band which comigrated with the *Hin*dIII-fragment of the N gene. These bands presumably were chimeric fragments resulting from integration of multiple copies of foreign DNA at various positions in the plant genome. Such bands may contain truncated and/or rearranged *Hin*dIII fragments of the N gene. In addition, the band patterns observed on the Southern blot indicated that the primary transgenic plants were derived from at least three independent transforma-

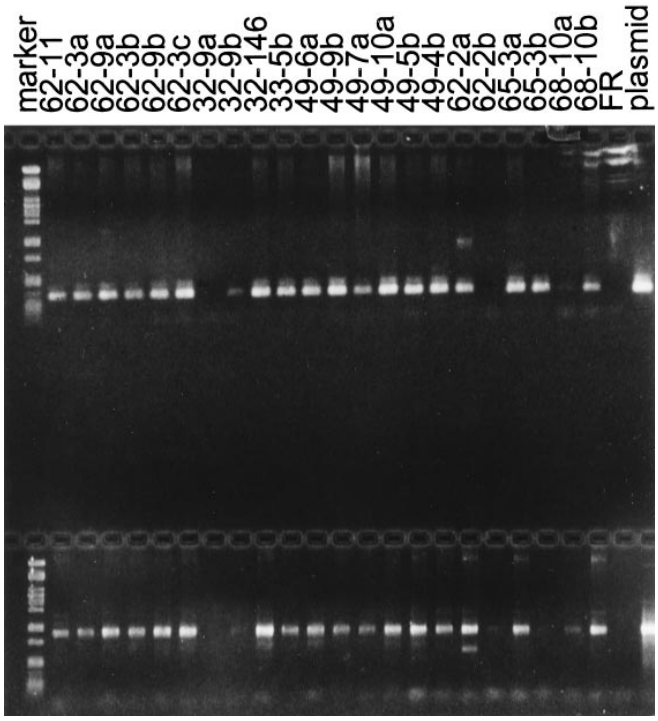

Fig. 1 PCR analysis of DNA from transgenic peanut plants. The amplification product shown in the upper row of lanes is the 380-bp fragment from the CaMV 35S promoter and the *hph* coding region. The amplification product in the lower row of lanes is the 950-bp fragment from the CaMV 35S promoter and the N coding region. The marker is *Pst*I-digested lambda DNA (*numbered lanes* represent transgenic plants, *FR* non-transgenic control Florunner, *plasmid* positive control with pCB 13-N^{+})

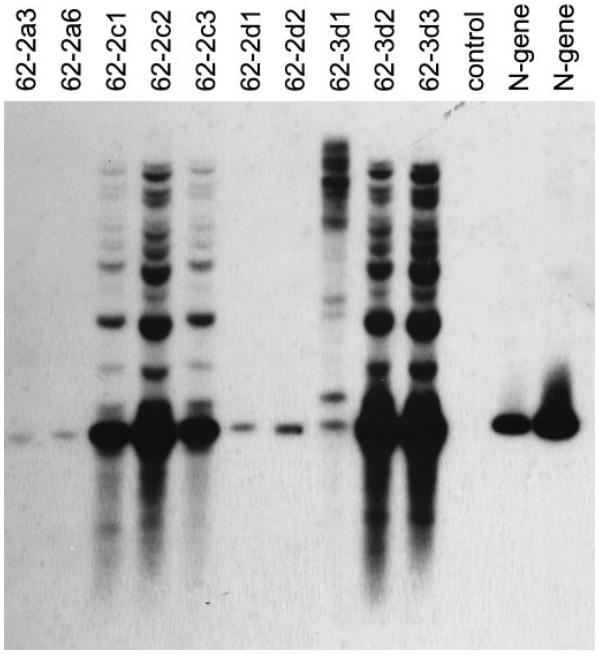

Fig. 2 Southern blot analysis of *Hin*dIII-digested genomic DNA from transgenic peanut plants using the ^{32}P-labeled TSWV N gene as a probe (*numbered lanes* represent transgenic plants, *control* non-transgenic peanut, *N-gene* 1.2-kb *Hin*dIII fragment of the TSWV N gene excised out of the vector pCB-N^{++})

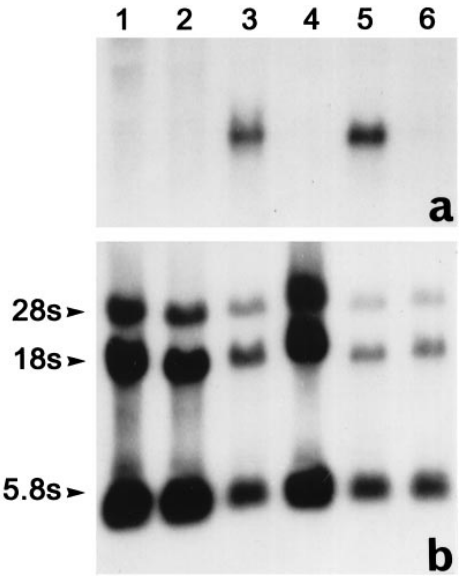

Fig. 3 Northern blot of TSWV N gene transcripts in transgenic peanut plants probed with the N gene (**a**) and reprobed with ribosomal DNA containing 28 s, 18 s, and 5.8 s sequences (**b**) (*lanes 1, 2, 4* RNA isolated from 62-3d1, 2, 3; *lanes 3, 5* RNA isolated from R_1 plant 62-2a3-2 and R_0 parent 62-2a3, respectively; *lane 6* RNA isolated from 62-2c2)

tion events. The transgenic plants 62-2c1, 62-2c2, 62-2c3, 62-3d2, and 62-3d3 showed the same banding pattern, suggesting that they may have originated from the same transgenic cell. The transgenic plant 62-3d1 showed a unique banding pattern and must have developed from a different transgenic cell line than all other plants tested. In comparison to other transgenic plants, 62-3d1 was morphologically distinct with a yellowish leaf color and extremely reduced growth in the greenhouse. Since multiple cell lines were occasionally recovered from a single flask at different times, it is possible that not all were of independent origin because of the fragmentation of embryogenic tissues growing under agitation in liquid medium. It might also be possible for an embryogenic clump to comprise a chimera of two different transgenic lines.

By Northern analysis, no transcripts of the N gene were detected in transgenic plants 62-3d1, 62-3d2, 62-3d3, and 62-2c2, although a relatively uniform signal from rRNA was observed (Fig. 3). Neither was N protein detected by ELISA in the leaf extract of 62-3d3. These plants were also in the group that showed strong hybridization signals with multiple bands on Southern blots. When total RNA samples from primary transgenic plant 62-2a3 and one of its progeny were included on a Northern blot, we observed a single hybridizing band of the expected size (1.3 kb) which represented the N gene transcript (Fig. 3), and this plant also showed only a single band by Southern analysis (Fig. 2). These data suggest that the absence of N gene transcripts in some of the transgenic plants may be correlated with the high transgene copy number in the host genome.

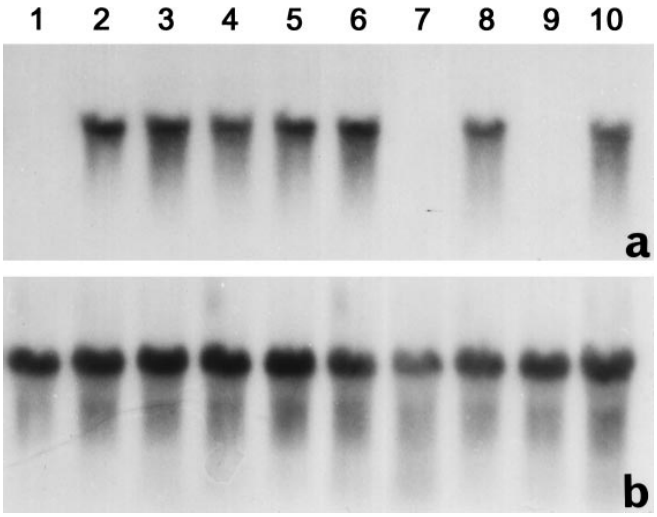

Fig. 4 Northern blot analysis of TSWV N gene expression in 10 R_1 plants of the transgenic line 62-2a6 probed with the N gene (**a**), and reprobed with 18 s rDNA (**b**). Positive ELISA results corresponded with N gene RNA expression

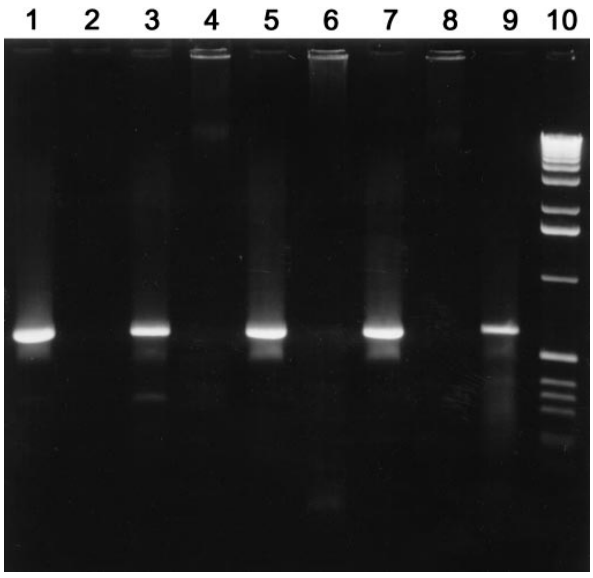

Fig. 5 Agarose gel electrophoresis of RT-PCR products obtained from mRNA of transgenic plants (*lanes 1, 3, 5, 7* RT-PCR products of progeny 62-2a6-3 through -6; *lanes 2, 4, 6, 8* PCR control reactions (no reverse transcription) for the same progeny; *lane 9* PCR product amplified from genomic DNA of 62-2a6; *lane 10* 1-kb DNA ladder

Of 60 progeny of 62-2a6 which were subjected to ELISA assay using an anti-TSWV-N protein antibody, N gene expression was detected in 43 plants, while 17 plants showed no reaction. Since peanut is a self-pollinated plant, the segregation of N gene expression in the R_1 progenies of the transgenic plant 62-2a6 followed the expected Mendelian segregation ratio (3:1; $\chi^2 = 0.356$, $P = 0.55$). The result suggested that only one copy of the N gene was integrated in the plant genome, or possibly several expressed copies could have been integrated in a cluster which behaved as a single locus. The progeny of 62-2a6 which showed a strong ELISA signal were also positive at the mRNA level, while the progeny which did not show any immunological color development were negative for mRNA of the N gene (Fig. 4). The OD_{490} values of 100-µl samples from the ELISA-positive plants ranged from 2.66 to greater than 4.00 while plants were considered negative for expression when the OD_{490} was less than 0.10. The positive TSWV-infected tobacco control showed an OD_{490} of 2.71. One positive control, a TSWV-infected plant of Florunner, failed to show an ELISA-positive signal which may have been due to sampling error since TSWV typically is not uniformly distributed throughout individual host plants (Kresta et al. 1995). In a repeated ELISA assay, a different leaf extract from the same infected plant did respond positively (data not shown).

Four of the Northern- and ELISA-positive progeny plants were also analyzed by RT-PCR using primers flanking a portion of the N gene coding sequence. A clear band of the expected size (0.62 kb) was amplified in all four plants tested (Fig. 5). The fact that three of the four control reactions which contained all the components for RT-PCR except reverse transcriptase failed to amplify, and only one produced a barely discernible DNA band (Fig. 5, lane 6) suggested that the products of the RT-PCR reactions were indeed amplified cDNAs synthesized from mRNA and not contaminating genomic DNA.

The production of transgenic plants has become routine for a number of crop species, including peanuts in our laboratory (Ozias-Akins et al. 1993; Singit et al. 1997; Wang et al. 1998). However, the levels of transgene expression generally are unpredictable and vary among independent transformants (Finnegan and McElroy 1994). Although the phenomenon is not completely understood, increasing numbers of parallel observations suggest that copy number and/or insertion site may be responsible (Flavell 1994; Meyer 1996). Linn et al. (1990) showed that in petunia plants transformed with a *Zea mays A1* gene, plants with single-copy insertions usually showed uniform and predictable expression of pigment, whereas transformants containing multiple copies of the gene generally exhibited methylation of all the promoter regions and showed no expression of the transgene. Integration of multiple copies of the GUS gene into tobacco led to lower expression than a single-copy insertion (Hobbs et al. 1990). Our data show another example of transgene inactivation correlated with multiple-copy insertions in the host genome, although we have not demonstrated whether inactivation was due to the absence of functional copies or a silencing mechanism. Distinguishing between transcriptional and post-transcriptional silencing would require the performance of nuclear run-on analysis (Dehio and Schell 1994).

Our expression analysis indicated that single- or low-copy-number insertions resulted in predictable expression in transgenic progeny, hence any means to reduce copy number of transgenes would be desirable if the goal is to achieve stable protein expression. Unfortunately, *Agrobacterium*-mediated transformation, which is known to pro-

duce a higher frequency of low-copy-number transformants than free DNA uptake methods, is highly genotype dependent in peanut (Cheng et al. 1996; Li et al. 1997); therefore, direct gene transfer remains the sole alternative method for transformation of runner-type peanut cultivars. Hansen and Chilton (1996) bombarded maize suspension cells using constructs having T-DNA border sequences codelivered on a separate plasmid with *vir* genes driven by the CaMV 35 S promoter. Sequence data showed that some inserts exhibited right border junctions with plant DNA that corresponded precisely to the sequence expected for T-DNA insertion events. This plant transformation technique, termed "agrolistic," combines the advantages of low-copy insertion of the *Agrobacterium* transformation system with the high efficiency of biolistic DNA delivery. Inclusion of nuclear matrix attachment regions (MAR) flanking a transgene has been shown to both increase the level and lower the variability of transgene expression by reducing the influence of enhancer and other cis-acting regulatory elements surrounding a given integration site (Breyne et al. 1992; Allen et al. 1993). Incorporation of MARs into transgene constructs could be one approach to stabilization of gene expression in peanut and other crops.

It has been shown in the literature that protection of transgenic plants against TSWV is under both RNA- and protein-mediated control (Pang et al. 1993). In *Nicotiana*, resistance to the tomato spotted wilt virus is, to a large degree, a consequence of the untranslated RNA transcripts (De Haan et al. 1992; MacKenzie and Ellis 1992; Pang et al. 1992, 1993, 1996, 1997; Kim et al. 1994; Vaira et al. 1995). When tobacco was transformed with a translationally defective or antisense gene, the transgene gave high levels of resistance to TSWV. The ratio of resistant plants was noted to be close to that obtained from tobacco plants containing a translationally intact gene.

If transgenic peanut containing the N gene reacts to TSWV infection in a manner similar to tobacco, we could predict that the line with high N protein expression would show some tolerance to TSWV (probably a delay in symptoms) and perhaps to other tospoviruses. Mechanical inoculation of peanuts is not highly reproducible, and although Li et al. (1997) observed a delay in symptom development in transgenic New Mexico Valencia A, this reaction did not result in useful field resistance. Stable, engineered resistance likely will require the production of numerous independent transformants to allow the selection of one with the appropriate level of N gene expression.

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