Sources of Resistance to *Tobacco streak virus* in Wild *Arachis* (Fabaceae: Papilionoidae) Germplasm

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ABSTRACT

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Stem necrosis disease caused by *Tobacco streak virus* (TSV), first recognized in 2000, has emerged as a potential threat to peanut (*Arachis hypogaea*) in southern states of India. The virus induces severe necrosis of shoots leading to death of the plant, and plants that survive are malformed, with severe reduction in pod yield. All the currently grown peanut cultivars in India are highly susceptible to the virus. Therefore, wild relatives of peanut were evaluated to identify potential sources of resistance to TSV infection. In all, 56 germplasm accessions from 20 wild *Arachis* spp. in four sections (*Arachis, Erectoides, Procumbente*, and *Rhizomatosae*), along with susceptible peanut cultivars (JL 24 and K 1375), were evaluated for resistance to TSV under greenhouse conditions using mechanical sap inoculations. Systemic virus infection, determined by enzyme-linked immunosorbent assay (ELISA), in the test accessions ranged between 0 and 100%. Twenty-four accessions in section *Arachis* that had 0 to 35% systemically infected plants were retested, and systemic infection was not detected in eight of these accessions in repeated trials in the greenhouse. These are International Crops Research Institute for the Semi-Arid Tropics groundnut (ICG) accession nos. 8139, 8195, 8200, 8203, 8205, and 11550 belonging to *A. duranensis*; ICG 8144 belonging to *A. villosa*; and ICG 13210 belonging to *A. stenosperma*. Even though the resistant accessions had 0 to 100% TSV infection in inoculated leaves, TSV was not detected in the subsequently emerged leaves. This is the first report of TSV resistance in *Arachis* spp. The eight TSV resistant accessions are cross compatible with *A. hypogaea* for utilization in breeding for stem necrosis disease resistance.

Additional keywords: *Ilarvirus*, thrips

Peanut or groundnut (*Arachis hypogaea* L.) is an important oil, food, and forage legume grown on 6.72 million ha in India (5). Nearly 11% of the total peanut production in India comes from the Anantapur district of Andhra Pradesh state, which has the distinction of being the world's largest peanut-producing region, with more than 70% of the cultivated area devoted to peanut production. The stem necrosis disease caused by *Tobacco streak virus* (TSV; genus *Ilarvirus* and family *Bromoviridae*) on peanut first was noticed in Anantapur district in 2000 (19,23). The virus epidemic that year affected 225,000 ha, resulting in yield losses valued at US$65 million (23). Since then, the TSV incidence has been monitored regularly in Andhra Pradesh and the adjoining regions in Karnataka state, with incidence ranging from 0 to 15% in 2002 to 0 to 3% in 2003 and 1 to 80% in 2004 (12,20). TSV infection at early stages of plant growth results in severe necrotic symptoms on leaves, petioles, and stems leading to premature death of the plant. Virus infection at later stages of plant growth (>40 days old) results in partial necrosis on leaves and main stem, proliferation of axillary buds, and drastic reductions in pod yield. Some plants may not become necrotic, but are severely stunted, having small leaves with or without chlorosis (23). The TSV isolate from peanut is serologically related to the TSV-WC strain, and shares high levels of nucleotide sequence identity (85 to 90%) with this strain in the movement and coat protein gene sequences (1,23). Three species of thrips, *Megalurothrips usitatus, Frankliniella schultzei*, and *Scirtothrips dorsalis*, transmitted the virus to peanut, apparently by mechanical inoculation in the presence of infected pollen rather than as direct vectors (20,25). The virus is not seedborne in peanut, and cleistogamous flowering prevents further spread of virus from peanut.

Since the first identification of TSV in 2000, the virus has been found on several vegetable and oilseed crops in Andhra Pradesh, Karnataka, Maharashtra, and Tamil Nadu states in India (7,11,12). The virus causes asymptomatic infections in several common weed species, including *Parthenium hysterophorus, Ageratum conyzoides,* and *Corchorus trilocularis,* whose pollen is a major source of TSV, and these plants also harbor thrips (20,21). Disease incidence in peanut and other susceptible crops is increasing at an alarming rate and is very high during low-rainfall and drought years. None of the peanut cultivars currently grown in India has resistance to TSV infection. Management strategies based on cultural practices, such as seed treatment with imidacloprid to control the thrips vector, barrier crops with fast-growing tall cereals to prevent insect movement, removal of TSV-susceptible weed hosts, and maintaining optimal plant density were shown to reduce disease incidence, but are seldom practiced under subsistence agriculture systems (21). In our earlier studies, none of the 150 peanut cultivars and advanced breeding lines evaluated for TSV resistance by sap inoculation were resistant (8,21). Symptom expression was delayed in three breeding lines, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) groundnut variety (ICGV) nos. 92267, 99029, and 01276, but they succumbed under high disease pressure (21). This situation necessitated a search for sources of durable resistance in wild *Arachis* germplasms, which are reported to carry genes for many agronomic traits as well as resistance genes against several pathogens and insect pests (9,14). Therefore, this study was conducted to evaluate 56 germplasm accessions from 20 wild *Arachis* spp. in four sections for TSV resistance under greenhouse conditions, and reports the occurrence of resistance to TSV in some accessions of the section *Arachis* that can be introgressed into elite peanut cultivars through interspecific breeding programs.

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MATERIALS AND METHODS

Seed material and plant growth conditions. Fifty-six wild *Arachis* accessions were obtained from the R. S. Paroda Gene Bank at ICRISAT, Patancheru, India (Table 1). Peanut cvs. JL 24 and K 1375 were used as susceptible controls. The 56 wild *Arachis* accessions were tested in batches from June to November 2004, along with susceptible controls. Promising accessions selected from this trial were reevaluated from September to November 2005, and again from May to July 2006, under similar plant growth conditions. Seed of each accession were treated with thiram (Chemet Chemicals Ltd., Gujarat, India) (3 mg g^{-1} of seed), sown in 22-cm-diameter plastic pots filled with sterilized Alfisols, and maintained in an insect-proof greenhouse (80% humidity and 25 to 30°C under natural light). The experiments were arranged in a randomized block design with two replications, with each pot containing three to seven seedlings considered as a replication.

Virus culture and inoculation. The TSV culture used in the study was isolated from diseased peanut plants collected in Anantapur during 2000, and subsequently established on *Phaseolus vulgaris* cv. French bean, which was lyophilized and preserved at –70°C (20). A week before inoculation, sap extracts from the lyophilized leaf material were inoculated onto cotyledons of French bean as described by Reddy et al. (23). Symptomatic leaves of these plants were ground in 0.05 M phosphate buffer (1:10 wt/vol) containing 0.15% monothioglycerol and the inoculum was applied onto Carborundum-dusted upper leaf surfaces of all the three quadrifoliate leaves of wild *Arachis* and control plants at a three-leaf growth stage. Inoculated leaves were washed with distilled water and kept in the dark for 12 to 14 h. Plants were monitored for TSV infection at 2-week intervals until the plants were 3 months old.

Detection of TSV. Polyclonal antibodies raised to TSV were used to assay all the inoculated and subsequently emerged leaves of all test plants by direct-antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) as described by Hobbs et al. (6). Briefly, test leaves were extracted in 0.1 M carbonate buffer, pH 9.5, (1:20 wt/vol), and 100 µl were loaded into wells of ELISA plates. TSV antiserum was used at a 1:15,000 dilution after cross-adsorption with healthy peanut leaf extract (1:20 wt/vol). Alkaline phosphatase (ALP)-labeled goat anti-rabbit immunoglobulin Gs (Sigma-Aldrich, St. Louis) at 1:5,000 dilution and paranitrophenyl phosphate at 0.5 mg ml^{-1} in 10% (vol/vol) diethanolamine buffer, pH 9.8, were used to detect antigen-antibody complexes. Optical density at 405 nm was measured in a Titertek Multiskan ELISA reader after 60 min. Readings were considered virus positive if the absorbance values of samples

Table 1. Responses of accessions of wild *Arachis* inoculated with *Tobacco streak virus* (TSV) under greenhouse conditions during June to November 2004

Section, species (ICG no.)[b]	No. of plants tested	Mean percent infection[a]	
		Inoculated leaves	Subsequently produced leaves
Arachis batizocoi			
8124	6	0 (0.01)	16.5 (0.17)
8209	9	0 (0.01)	0 (0.01)
8210	7	100 (1.38)	100 (1.38)
13160	11	91 (1.14)	100 (1.34)
A. benensis			
13257	15	13 (0.13)	13 (0.13)
A. cardenasii			
11559	8	75 (0.85)	75 (0.85)
11561	7	71 (0.79)	71 (0.79)
11562	5	25 (0.27)	25 (0.27)
11563	12	83 (0.98)	83 (0.98)
11564	6	16.5 (0.17)	16.5 (0.17)
11566	10	20 (0.2)	10 (0.11)
12165	10	0 (0.01)	0 (0.01)
A. correntina			
8132	14	79 (0.91)	79 (0.91)
A. duranensis			
8123	10	0 (0.01)	10 (0.11)
8139	10	10 (0.11)	0 (0.01)
8195	8	0 (0.01)	0 (0.01)
8196	8	88 (1.08)	88 (1.08)
8199	10	100 (1.35)	70 (0.78)
8200	10	0 (0.01)	0 (0.01)
8201	9	89 (1.10)	89 (1.10)
8202	6	100 (1.40)	83 (098)
8203	10	0 (0.01)	30 (0.31)
8204	5	100 (1.41)	100 (1.41)
8205	10	0 (0.01)	0 (0.01)
8208	6	50 (0.52)	100 (1.40)
11550	10	0 (0.01)	0 (0.01)
11552	5	0 (0.01)	0 (0.01)
11554	8	63 (0.68)	38 (0.39)
13200	7	46 (0.49)	46 (0.49)
A. helodes			
8952	6	83 (0.98)	67 (0.73)
A. kempff-mercadoi			
8959	5	100 (1.41)	80 (0.93)
A. kuhlmannii			
8192	5	80 (0.93)	100 (1.41)
A. ipaensis			
8206	7	100 (1.38)	71 (0.79)
A. monticola			
8135	13	100 (1.32)	85 (1.02)
8197	6	100 (1.40)	100 (1.40)
8198	11	100 (1.34)	100 (1.34)
A. stenosperma			
8126	5	80 (0.93)	40 (0.41)
8137	6	0 (0.01)	16.5 (0.17)
13171	7	16.5 (0.17)	0 (0.01)
13172	9	45 (0.47)	12.5 (0.13)
13188	7	86 (1.04)	86 (1.04)
13210	6	0 (0.01)	0 (0.01)
13223	10	33.5 (0.37)	33.5 (0.37)
13233	6	16.5 (0.17)	33 (0.34)
A. villosa			
8144	7	54 (0.59)	0 (0.01)
13168	7	12.5 (0.13)	0 (0.01)
13259	8	25 (0.25)	38 (0.39)
A. rigonii			
8186	9	56 (0.59)	22 (0.22)
Arachis (species unknown)			
4982	12	100 (1.39)	58 (0.62)
Procumbentes appressipila			
8128	12	100 (1.33)	92 (1.12)

(*continued on next page*)

[a] Percent infection based on virus detection in enzyme linked-immunosorbent assay. Numbers in parenthesis = angular transformed values.
[b] ICG = International Crops Research Institute for the Semi-Arid Tropics groundnut accession number.
[c] SEM = standard error of the mean.
[d] LSD = least significant difference.

Table 1. (*continued from preceding page*)

Section, species (ICG no.)[b]	No. of plants tested	Mean percent infection[a]	
		Inoculated leaves	Subsequently produced leaves
8129	5	40 (0.41)	40 (0.41)
P. rigonii			
8904	5	60 (0.64)	100 (1.41)
P. kretschmeri			
8191	8	100 (1.37)	88 (1.08)
Erectoides paraguariensis			
8141	14	100 (1.31)	86 (1.04)
E. stenophylla			
8215	10	100 (1.35)	100 (1.35)
Rhizomatosae glabrata			
8937	9	100 (1.36)	100 (1.36)
Control (*A. hypogaea*)			
JL 24	10	40 (0.41)	100 (1.41)
K 1375	10	50 (0.53)	70 (0.79)
SEM (±)[c]	…	–0.0624	–0.0582
LSD (P = 5%)[d]	…	–0.175	–0.163

Table 2. Response of selected wild *Arachis* accessions for resistance to *Tobacco streak virus* (TSV) under greenhouse conditions during September to November 2005

Section, species (ICG no.)[b]	No. of plants tested	Mean percent infection[a]	
		Inoculated leaves	Subsequently produced leaves
Arachis batizocoi			
8124	8	100 (1.5)	75 (0.98)
8209	2	100 (1.5)	50 (0.75)
A. cardenasii			
11562	5	100 (1.46)	25 (0.27)
11564	9	90 (1.18)	77.5 (0.89)
11566	10	90 (1.17)	60 (0.64)
12165	7	29 (0.3)	87.5 (1.21)
A. duranensis			
8123	10	100 (1.41)	80 (0.93)
8139	4	50 (0.52)	0 (0.01)
8195	9	57.5 (0.63)	0 (0.01)
8200	9	35 (0.36)	0 (0.01)
8203	9	90 (1.18)	0 (0.01)
8205	7	60 (0.82)	12.5 (0.13)
11550	7	29 (0.3)	0 (0.01)
A. stenosperma			
8137	6	66.5 (0.89)	100 (1.51)
13171	10	80 (0.93)	70 (0.79)
13172	10	40 (0.41)	40 (0.41)
13210	7	46 (0.49)	0 (0.01)
13223	7	29 (0.3)	29.2 (0.3)
13233	4	50 (0.52)	0 (0.01)
A. villosa			
8144	4	0 (0.01)	0 (0.01)
13168	4	25 (0.26)	50 (0.52)
Control (*A. hypogaea*)			
JL 24	10	70 (0.79)	90 (1.25)
K 1375	10	70 (0.79)	50 (0.53)
SEM (±)[c]	…	(0.22)	(0.21)
LSD (P = 5%)[d]	…	(0.64)	(0.64)

[a] Percent infection based on virus detection in enzyme linked-immunosorbent assay. Numbers in parenthesis = angular transformed values.
[b] ICG = International Crops Research Institute for the Semi-Arid Tropics groundnut accession number.
[c] SEM = standard error of the mean.
[d] LSD = least significant difference.

were threefold higher than those of the healthy control samples. ELISA tests for TSV in inoculated leaves were done a week after inoculation, and tests were repeated at 2-week intervals in newly emerged leaves until the plants were 3 months old. Individual replicated observations were converted to percentages and subjected to angular transformation by substituting 0% by 1/4n and 100% by 100 – 1/4n, where n is the plant number. Data were subjected to analysis of variance using the GenStat 9.1 statistical package (Lawes Agricultural Trust, Rothamsted Experimental Station, England). Least significant differences at a 5% level of significance were used to test the differences among accessions.

RESULTS

The inoculated leaves of the 56 *Arachis* accessions developed chlorotic or necrotic patches and tested positive to TSV in ELISA (*data not shown*). The susceptible wild *Arachis* accessions developed systemic symptoms within 20 to 30 days post inoculation. Symptoms on these accessions were similar to those in cultivated peanut, such as leaf chlorosis followed by necrosis of leaves, petioles, and stems, and premature death of plants within 6 weeks post inoculation. Asymptomatic infection, delayed symptom expression, or symptom remission was not observed in the tested wild *Arachis* accessions. Percent infection in controls as well as test accessions reached a maximum level by 60 days after inoculation. In ELISA, only symptomatic plants were positive for TSV and all the asymptomatic plants were negative. The susceptible controls (peanut cvs. JL 24 and K 1375) had 50 to 100% infection, and typical TSV symptoms developed 12 to 20 days post inoculation (Tables 1, 2, and 3). The absorbance at 405 nm values of TSV-positive samples ranged between 1.5 and 3.0 and were less than 0.3 for uninfected and healthy control plants (*data not shown*).

Of the 56 accessions initially evaluated, 12 had no systemic infection (Table 1). Eight of these accessions (ICRISAT groundnut accession [ICG] nos. 8195, 8200, 8205, 8209, 11550, 11552, 12165, and 13210) were completely free from infection in both inoculated and subsequently emerged leaves. Virus was detected in inoculated leaves of four accessions (ICG nos. 8144, 8139, 13171, and 13168), but not in subsequently emerged leaves (Table 1). Among the remaining 44 accessions, infection in inoculated and systemic leaves ranged between 0 and 100% (Table 1). In four accessions (ICG nos. 8123, 8124, 8137, and 8203) virus was not detected in inoculated leaves; however, systemic infection was observed in 10 to 30% of these plants. This indicates a lack of correlation between virus in inoculated leaves and systemic infection. Moreover, the reactions of some genotypes were not consistent during reevaluations despite using similar plant growth and inoculation conditions (Table 2). For instance, during the first trial, ICG nos. 8124, 8137, and 12165 had less than 16% systemic infection whereas, in the second trial, the infection rate was greater than 70%, (Tables 1 and 2). Because of this situation, emphasis was placed on repeated testing of promising accessions in order to select accessions that had consistent resistance to systemic TSV infection.

All the accessions that had less than 35% systemically infected plants in the 2004 experiment were reevaluated during 2005; however, ICG nos. 8186, 13257, and 11552 were not retested due to nonavailability of seed (Table 2). All the resistant

accessions belong to section *Arachis*. Between 0 and 100% of the plants in each of the 21 accessions that were evaluated during 2005 became systemically infected (Table 2). None of the plants of ICG nos. 8139, 8144, 8195, 8200, 11550, and 13210 had systemic infections in either 2004 or 2005 (Tables 1 and 2). In addition, systemic infection was not observed in ICG nos. 8203 and 13233, which had 30 to 33% systemically infected plants in 2004. In the 2005 trial, only ICG no. 8144 was free of TSV infection in both inoculated and subsequently emerged leaves. Surprisingly, accessions ICG nos. 8209, 12165, 13171, and 13168, which had no systemic infection during the earlier trial, had 50 to 87.5% systemically infected plants in the second screening (Table 2).

Seven accessions (ICG nos. 8139, 8195, 8200, 8203, 8205, 11550, and 13210) that had 0 to 35% infection in the first two trials were evaluated during 2006 (Table 3). ICG 8144 and ICG 13233, which had 0 to 33% systemic infection during the first two trials, were not tested due to seed limitation. TSV was not detected in the systemic leaves of any of these accessions even though TSV was detected in 24 to 81% of inoculated leaves. The lack of systemic infection in repeated trials, despite the fact that some of these plants were positive to TSV infection in inoculated leaves, demonstrated consistent systemic resistance reactions to TSV (Table 3).

DISCUSSION

The viruses that have been recognized as economically important on peanut worldwide are *Cucumber mosaic virus, Cowpea mild mottle virus, Groundnut ringspot virus, Peanut bud necrosis virus* (PBNV), *Peanut stripe virus, Peanut clump virus, Indian peanut clump virus, Peanut mottle virus, Peanut stunt virus, Tomato spotted wilt virus* (TSWV), and groundnut rosette disease virus complex (22). Although TSV occurrence on peanut has been reported from Brazil (4) and South Africa (2), the virus was less prevalent and less important in Brazil, and was detected on only two peanut plants in South Africa, where it was described to cause chlorosis and malformed growth of young leaves (2). In contrast, TSV has emerged as a major production constraint on peanut in India. Reasons for such severe TSV epidemics are not known. Due to lack of effective TSV resistance in peanut cultivars, wild *Arachis* spp. were evaluated to identify sources of resistance that could be used in breeding programs.

The genus *Arachis* consists of 69 species placed under nine sections (10). The ICRISAT gene bank holds 452 accessions of 42 wild *Arachis* spp. representing eight sections (9,14). In the present study, 56 wild accessions were evaluated for TSV resistance (Table 1), followed by reevaluation of accessions that had lower incidence of systemic infection. Eight TSV resistant accessions were identified: ICG nos. 8139, 8200, 11550, 8195, 8203, and 8205 belonging to *A. duranensis*; ICG 13210 belonging to *A. stenosperma*; and ICG 8144 belonging to *A. villosa*. Although these genotypes are yet to be evaluated under field conditions in TSV-endemic areas, we hypothesize that the resistance will be effective against natural infection because the accessions were challenged with a high dosage of virus inoculum. Moreover, selection of genotypes with TSV resistance based on the genotype performance under natural occurrence of the disease in the field was precluded by the fact that TSV occurrence is sporadic and difficult to predict under the field situations. Our previous field trials to identify TSV resistance in peanut varieties, conducted during the 2001–03 rainy seasons (June to November) at Kadiri and Raichur, Andhra Pradesh, were inconclusive due to low natural incidence in the experimental plots (R. D. V. J. Prasadarao, *unpublished data*). Under such conditions, all plants may not be exposed to TSV inoculum; therefore, the level of true resistance cannot be assessed based on field observations only. Several studies have shown that experimental transmission of virus by mechanical sap inoculation or using viruliferous vectors under greenhouse conditions is effective to evaluate the level of resistance in a genotype by ensuring uniform inoculum pressure and growth conditions (13,16,24). In addition, Mandal et al. (16) reported that genotypes such as Georgia Green that are susceptible to TSWV by mechanical inoculation showed effective resistance against thrips-borne inoculum under field conditions. Reddy et al. (24) reported that, of seven wild *Arachis* accessions that were field resistant to thrips-borne PBNV inoculum, only three accessions were resistant to PBNV when tested by mechanical sap inoculations under greenhouse conditions. These examples indicate the robustness of the mechanical inoculation method for evaluating virus resistance in peanut genotypes.

We found that mechanical transmission of TSV from peanut to peanut was relatively difficult and often resulted in a significant number of escapes. A similar situation was reported with TSWV transmission from peanut to peanut (15). To enhance the transmission efficiency, we investigated the factors influencing the mechanical transmission of TSV to peanut and found that (i) serial passage of virus by mechanical transmission onto peanut or other susceptible herbaceous hosts resulted in attenuation of symptoms; (ii) transmission of TSV from peanut to peanut resulted in less than 60% infection, but 70 to 100% infection was obtained when the virus was

Table 3. Response of *Tobacco streak virus* (TSV)-resistant wild *Arachis* accessions to artificial inoculation with TSV under greenhouse conditions during May to July 2006

	Trial I[a]			Trial II[a]		
		Mean percent infection			Mean percent infection	
Section, species (ICG no.)[b]	*n*	Inoculated leaves	Subsequently produced leaves	*n*	Inoculated leaves	Subsequently produced leaves
Arachis duranensis						
8139	10	72 (0.88)	0 (0.009)	8	0 (0)	0 (0)
8195	17	36 (0.37)	0 (0.003)	NT	NT	NT
8200	19	29 (0.3)	0 (0.003)	9	55 (0.58)	0 (0)
8203	10	23.75 (0.25)	0 (0.002)	NT	NT	NT
8205	16	23.5 (0.24)	0 (0.002)	NT	NT	NT
11550	17	43.75 (0.46)	0 (0.004)	6	0 (0)	0 (0)
A. stenosperma						
13210	16	81 (1)	0 (0.009)	NT	NT	NT
A. hypogaea						
JL 24	20	67.5 (0.75)	60 (0.007)	20	40 (0.416)	80 (0.937)
SEM (±)[c]	…	(0.12)	(0.001)	…	(0.0432)	(0.0353)
LSD (P = 5%)[d]	…	(0.35)	(0.003)	…	(0.1381)	(0.1128)

[a] Percent infection based on virus detection in enzyme-linked immunosorbent assay, n = number of plants tested, number in parenthesis = angular transformed values, and NT = not tested.
[b] ICG = International Crops Research Institute for the Semi-Arid Tropics groundnut accession number.
[c] SEM = standard error of the mean.
[d] LSD = least significant difference.

transmitted from French bean to peanut; (iii) use of various combinations of antioxidants, abrasives, and inoculation methods did not significantly affect infectivity; and (iv) day temperatures of greater than 40°C (April to May) and night temperatures of less than 15°C (December) significantly reduced infectivity and symptom expression (*data not presented*). Based on these observations, the mechanical sap inoculation conditions (described in this study) were optimized for evaluation of TSV resistance in the *Arachis* germplasm by administering a large dosage of virus inoculum under greenhouse conditions. This procedure usually resulted in more than 80% transmission rate in highly susceptible cultivars such as JL 24 (Tables 1, 2, and 3). In the susceptible cv. K 1375, only 50 and 70% of the plants were infected in two experiments (Tables 1 and 2). However, this accession was regarded as highly susceptible to TSV as per the criteria set in this study (>35% infected plants). The most likely explanation for the variability in TSV transmission rates of highly susceptible cultivars such as JL 24 and K 1375, and even in wild *Arachis* accessions could be escapes. For this reason, emphasis has been placed on repeated testing of promising accessions to assess the virus resistance. All eight resistant accession selected had consistent resistant responses across all three trials.

Differences in virus infection in the inoculated leaves and subsequently emerged leaves was observed in susceptible controls and test plants. On a few occasions, higher incidence of systemic infection compared with the inoculated leaves was observed. For instance, virus was detected in the inoculated leaves of 40 and 50% of the systemically infected JL 24 and ICG 8208, respectively (Table 1). This could be due to low virus concentration in the inoculated leaves that were undetectable by ELISA at 1 week post inoculation. This is likely because virus was detected in such leaves when retested 2 weeks after inoculation (*data not presented*). However, at 2 weeks post inoculation, inoculated leaves of most of the test plants were senesced or damaged due to necrosis, precluding routine testing. In most cases, the incidence of infection in inoculated and systemic leaves was similar (100% infection in ICG nos. 8135, 8198, and 11563; 16.5% in 11564; and 80% in 11563; Table 1) or higher in inoculated leaves (ICG nos. 8206 and 8199; Table 1); therefore, the data suggest that virus reaches adequate concentration in inoculated leaves by 1 week post inoculation to provide for systemic movement in susceptible accessions.

Accessions from the same species varied in their reactions to TSV. For instance, 6 of 16 *A. duranensis* accessions evaluated were resistant to TSV (Table 1). These differences in disease reaction may be due to genetic variation within the species, as has been observed earlier in wild *Arachis* spp. (18,24,27). In most of the resistant accessions, virus multiplication was detected in inoculated leaves, suggesting that the resistance mechanism results from a block to systemic spread of the virus. Grafting experiments, which were not done in this study, could provide information on whether any of these genotypes were immune to TSV infection (3). A similar mechanism (that is, lack of systemic spread of virus from inoculated leaves in spite of repeated mechanical sap inoculations) was reported for PBNV resistance in wild *Arachis* germplasm (24).

The resistant accessions ICG nos. 8139 and 11550 also possess high levels of resistance to rust (*Puccinia arachidis*) and late leaf spot (*Phaeoisariopsis personata*) (18) and ICG no. 8144 to *Peanut bud necrosis virus* (24) Thus, these accessions possess resistance to multiple pathogens and might be used to develop multiple disease-resistant peanut cultivars through interspecific breeding programs. All the TSV-resistant accessions are in section *Arachis*, have an 'A-genome' in common with the cultivated peanut, and are cross compatible with *A. hypogaea*. Seed of resistant accessions are available at the ICRISAT gene bank for utilization in breeding programs. Earlier studies have used accessions from section *Arachis* in conventional breeding programs to transfer resistance to rust and late leaf spot into agronomically elite cultivars (17,26). Therefore, there is a potential to transfer TSV resistance into widely adapted peanut cultivars. Work has been initiated at ICRISAT to develop interspecific hybrids using ICG nos. 8139 and 8144 for TSV resistance. To our knowledge, this is the first report of identification of resistance to TSV in wild *Arachis* germplasm.

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