

Evaluation of peanut genotypes for resistance to *Tomato spotted wilt virus* by mechanical and thrips inoculation

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Abstract – The objective of this work was to evaluate the reactions of three peanut breeding lines (IC-10, IC-34, and ICGV 86388) to *Tomato spotted wilt virus* (TSWV) by mechanical and thrips inoculation, under greenhouse conditions, and compare them to the reactions of cultivars SunOleic, Georgia Green, and the breeding line C11-2-39. TSWV infection by mechanical inoculation was visually assessed using an index ranging from 0 (no symptoms) to 4 (apical death). Enzyme-linked immunosorbent assay was used to confirm TSWV infection from both mechanical and thrips inoculations. IC-10, IC-34, ICGV 86388, and C11-2-39 were more resistant than the cultivars SunOleic and Georgia Green based on mechanical inoculation. Upon thrips inoculation only IC-34 and ICGV-86388 were infected by TSWV, as demonstrated by reverse transcription polymerase chain reaction (RT-PCR), although no symptoms of infection were observed. The peanut breeding lines IC-10, IC-34, and ICGV 86388 show higher level of resistance to TSWV than cultivar Georgia Green considered a standard for TSWV resistance.

Index terms: *Arachis hypogaea*, TSWV, mechanical transmission, thrips transmission.

Avaliação de genótipos de amendoim em relação à resistência ao *Tomato spotted wilt virus* por meio de inoculação mecânica e por tripses

Resumo – O objetivo deste trabalho foi avaliar as reações das linhagens de amendoim IC-10, IC-34 e ICGV 86388 quanto à resistência ao *Tomato spotted wilt virus* (TSWV), por meio de inoculação mecânica e por tripses, em casa de vegetação, e compará-las às reações das cultivares SunOleic, Georgia Green e da linhagem C11-2-39. A infecção de TSWV após inoculação mecânica foi visualmente avaliada, utilizando-se escala de notas que variam de zero (sem sintomas) a quatro (morte apical). A análise pelo teste ELISA confirmou a infecção de TSWV em ambos os tipos de inoculação. As linhagens IC-10, IC-34, ICGV 86388 e C11-2-39 foram mais resistentes do que as cultivares SunOleic e Georgia Green, com base nos resultados da inoculação mecânica. Em relação à inoculação por tripses, apenas IC-34 e ICGV 86388 foram infectadas por TSWV, conforme demonstrado pela reação em cadeia da polimerase com transcrição reversa (RT-PCR), embora nenhum sintoma de infecção tenha sido observado. As linhagens IC-10, IC-34 e ICGV 86388 mostram maior resistência ao vírus, quando comparadas à Georgia Green, considerada padrão de resistência ao TSWV.

Termos para indexação: *Arachis hypogaea*, TSWV, transmissão mecânica, transmissão por tripses.

Introduction

Peanut (*Arachis hypogaea* L.) is an important oilseed crop throughout the world, and one of the main limiting factors to its production is the infection by *Tomato spotted wilt virus* (TSWV) (Culbreath et al., 2003). Tomato spotted wilt disease was first described in Australia on tomato (*Lycopersicon esculentum* Miller) (Brittleblank, 1919), and its viral etiology was confirmed

in 1930 (Samuel et al., 1930). TSWV is capable of infecting more than 800 species of plants, monocot and dicot, within more than 70 families (Goldbach & Peters, 1994). Epidemics of TSWV occur in many parts of the world causing losses in several crops (Culbreath et al., 2003).

TSWV is the type member of the genus *Tospovirus* in the family *Bunyaviridae* (Van Regenmortel et al., 2000). The virions are spherical enveloped particles,

80–110 nm in diameter. TSWV is transmitted by several species of thrips (Thysanoptera: Thripidae) in a persistent, propagative manner (German et al., 1992). Thrips that acquire TSWV at the larval stage are able to transmit the virus after an incubation period. Both larvae and adults, derived from immature thrips that acquired the virus, may transmit TSWV (Ullman et al., 1992). The tobacco thrips (*Frankliniella fusca* Hinds) is the most important vector of TSWV in peanut in Georgia (Brown et al., 2003). TSWV is also readily transmitted by mechanical inoculation.

Effective management of tomato spotted wilt disease has been obtained through strategy combining resistant cultivars with cultural methods such as planting date, plant population, insecticide usage, row pattern, tillage, and herbicide (Brown et al., 2003; Culbreath et al., 2003). None of these cultural management parameters are efficient in TSWV incidence reduction without the cultivar resistance, and new sources of resistance are necessary for effective long-term disease management (Brown et al., 2003).

The objective of this work was to evaluate the reactions of three peanut genotypes under greenhouse conditions, and to compare their response to TSWV by mechanical and thrips inoculation.

Material and Methods

A previously described TSWV isolate (Pappu et al., 1998) was maintained on *Emilia sonchifolia* L., under greenhouse conditions, by thrips transmission using *Frankliniella fusca* (TT) and *F. occidentalis* Pergande (western flower thrips – WFT). Symptomatic plants were tested by enzyme-linked immunosorbent assay (ELISA) to confirm TSWV infection. Cultures of virus-free thrips were reared and maintained in the laboratory on green bean pods as previously described by Ullman et al. (1992) and Assis Filho et al. (2002).

The peanut breeding lines IC-10, IC-34, and ICGV-86388 were tested for TSWV resistance and showed field resistance to the TSWV-related tospovirus *Peanut bud necrosis virus* under natural infection in Thailand (Pensuk et al., 2002). The cultivar Georgia Green and the breeding line C11-2-39 were used as standards for resistance (Culbreath et al., 1996, 1999, 2003; Brown et al., 2003), and cultivar SunOleic as standard for susceptibility (Culbreath et al., 1999).

Peanut seeds were germinated in a growth chamber at 25°C, 16-hour day length. Plants at the four-leaf stage

were placed in the dark for approximately five hours before inoculation. Inoculum was prepared by grinding 1 g of leaf tissue from systemically infected *E. sonchifolia* in 10 mL of 0.05 M sodium phosphate buffer, pH 7, containing 0.2% 2-mercaptoethanol and 1% celite. The inoculum was kept on ice and replaced with freshly ground leaf tissue every 30 minutes. Inoculum was applied to Carborundum-dusted leaves and rubbed on both surfaces of the lamina.

After inoculation, plants were sprayed with distilled water and kept in the growth chamber at 25°C, 16-hour day length. Each treatment was composed by cultivars SunOleic and Georgia Green, and breeding lines C11-2-39, IC-10, IC-34, and ICGV 86388 with ten replicates, represented by individual plants. Treatments were arranged in a completely randomized design.

The experiment was repeated three times. In each repetition, ten seedlings of each genotype were inoculated as described above. The seedlings inoculated were observed for a 25-day period for symptom development, when seedlings were individually inspected in a daily basis, and assigned a disease index from 0 to 4 for statistical analysis, in which: 0 corresponds to no symptoms; 1, chlorotic or necrotic local lesion; 2, mosaic; 3, leaf distortion; and 4, apical death.

For each experiment, the disease indexes of the ten seedlings within a given treatment were added together and used to calculate the average for a given day. Finally, the daily average for each experiment was used to calculate the overall average and subsequently plotted to generate the disease progression curve (Figure 1).

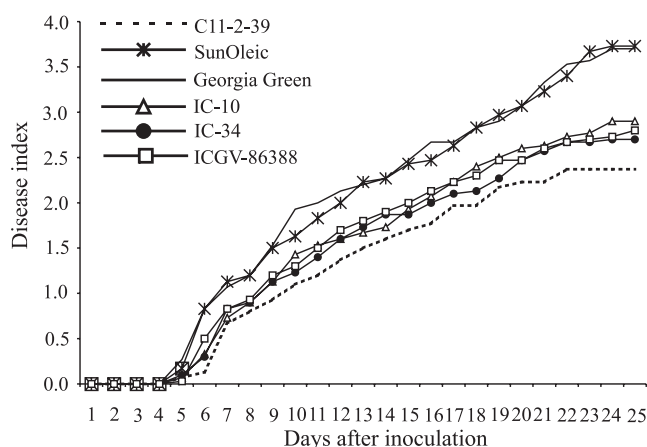


Figure 1. Disease progression curves of *Tomato spotted wilt virus* (TSWV) in peanut genotypes. Values plotted are means of ten replications per experiment pooled over three experiments.

The statistical analysis was done on the means of the disease indexes from 10 replications for each treatment, using the ANOVA procedure of SAS (SAS Institute, Cary, NC), at the probability level of 5%, by the F test.

Seedlings infection was verified 10 and 25 days after inoculation through ELISA test, using samples of leaves, and leaves and roots, respectively. Double antibody sandwich-ELISA (DAS-ELISA) was used to detect TSWV infection using a commercial kit (Agdia, Elkhart, IN), according to manufacture instructions. Samples were considered positive if absorbance readings (405 nm) were greater than three times those of healthy control plants, using an ELISA plate reader.

For thrips inoculation, peanut seeds were germinated in a greenhouse in thrips-proof cages. Viruliferous thrips were obtained from a colony that acquired the virus as following. First instar larva of TT, up to 24-hour old, were given a 24-hour acquisition access period by feeding on excised leaves of SWV-infected *E. sonchifolia*. After the acquisition access period, the insects were transferred to green bean pods and reared to adults. A sample of 24, 8 to 9-day old adult thrips was assayed by immunofluorescence microscopy (Assis Filho et al., 2002) to verify acquisition. Afterwards, adult thrips were confined for a 5-day inoculation access period on individual seedling of each peanut genotype, at the stage in which the first trifoliate leaf was just opened. It was used 30 thrips per seedling.

The plants were kept in the greenhouse, inside the cage, and were observed during 30 days after inoculation access period for visual symptoms, then tested by ELISA and reverse transcription-polymerase chain reaction (RT-PCR) for virus infection. Each treatment was composed by cultivars SunOleic and Georgia Green, and breeding lines C11-2-39, IC-10, IC-34, and ICGV 86388 with 6 replicates, represented by individual plants. The experiment, as described above, was repeated twice. The evaluation was expressed as the proportion between the number of plants tested positive and the number of plants tested negative by RT-PCR, pooling the total number of plants within each treatment from the 3 experiments. Total RNA, extracted from all plants submitted to thrips inoculation, was performed using the RNeasy Mini Kit. A pair of primers (5'-CATGTCTTCAA GTGTTTATGAGTC-3', position 89 to 111 and

5'-CGAGTTATTTTGATCCTGAAGCAT-3', complementary to position 1483 to 1464) was designed to amplify a 1,395 bp fragment of TSWV S RNA (GenBank Accession number D00645). RT-PCR was done (Deom et al., 2000) using 100 µL of reaction with denaturation at 94°C for 1 minute, annealing at 55°C for 2 minutes, and extension at 72°C for 5 minutes, for a total of 30 cycles. Five microliters of PCR products were analyzed by 0.8% agarose gel electrophoresis and stained with ethidium bromide.

Results and Discussion

Plants from all peanut genotypes tested became systemically infected with TSWV by mechanical inoculation. The breeding lines IC-10, IC-34, ICGV 86388 and C11-2-39 presented higher resistance to TSWV than the cultivar Georgia Green, the standard for resistance used in this study (Figure 1).

The disease index indicated that the breeding lines IC-10, IC-34, ICGV 86388 and C11-2-39 did not express stunting or apical death. Infection was confirmed by DAS-ELISA (Table 1). Generally, the first symptoms appeared between 5 and 7 days after inoculation and included necrotic and chlorotic local lesions. Subsequently, plants showed mosaic, foliar distortion, stunting, and apical death. The symptom severity progressed similarly in all genotypes tested, except for

Table 1. Reaction of *Arachis hypogaea* genotypes to *Tomato spotted wilt virus* (TSWV) by mechanical inoculation, evaluated through enzyme-linked immunosorbent assay (ELISA) and symptomatology.

Genotype	Tissue used for ELISA ⁽¹⁾		Index disease ⁽²⁾	Infected plants (%) ⁽³⁾
	Leaves	Roots		
SunOleic	0.954	1.062	4	75.0a
Georgia Green	1.026	1.337	4	74.8a
IC-34	0.760	0.882	3	50.0ab
C11-2-39	0.873	0.971	3	45.0b
ICGV-86388	0.836	0.824	3	42.7b
IC-10	0.726	0.876	3	34.1b
Healthy control	0.121	0.207	0	0

⁽¹⁾Mean of ELISA values with absorbance at 405 nm; each sample was tested in three replicated wells. ⁽²⁾Symptoms evaluated during 25 days after TSWV inoculation: 0 (no symptoms), 1 (chlorotic or necrotic local lesion), 2 (mosaic), 3 (leaf distortion) and 4 (stunting or apical death); values represent the maximum disease index observed in each genotype. ⁽³⁾Means followed by different letters are significantly different at the probability level of 5%, by the F test.

Georgia Green and SunOleic, which exhibited stunting or apical death.

The percentage of plants determined to be infected based on ELISA, using roots and leaves 25 days after inoculation, was significantly different by F test at the probability level of 5% (Table 1). The breeding lines C11-2-39, ICGV 86388 and IC-10 showed lower percentage of infection than the breeding line IC-34, which showed a lower percentage of infection than the cultivars SunOleic and Georgia Green.

None of the plants inoculated by thrips showed visible symptoms in the 30 days after exposure to viruliferous thrips. Analysis by ELISA yielded no positive plants out of 72 tested. However, the viral S RNA was detected in 17 out of the 72 plants analyzed by RT-PCR. TSWV genome was detected in the breeding lines IC-34 (11) and ICGV-86388 (6), but not in the other genotypes.

Unsuccessful virus detection following experimental inoculation on susceptible host may have several origins. Under conditions similar to that studied here, the most common are escape and subliminal infection.

Escape is related to a combined effect of inoculation method and host response determined by the interaction host/vector/virus that does not favor virus infection. To minimize the escape effect, a high number of thrips per plant and a long inoculation access period were used in the experiments. Those factors, combined with the use of a thrips population showing a high percent of viruliferous individuals, as verified by immunofluorescence microscopy, assured the effectiveness of the inoculation method. Furthermore, thrips feeding activity was confirmed by the typical feeding scars observed on all seedlings subjected to the inoculation.

Subliminal infection refers to undetected virus replication due to abnormal virus replication and movement (Hull, 2002). Highly sensitive assay, such as RT-PCR, may detect virus replication in subliminal infection (Webster et al., 2004). It explains virus detection by RT-PCR but not by DAS-ELISA. Either escape or subliminal infection (also called field resistance) may represent a resistance strategy that differentiates the genotypes evaluated.

There are differences in the reaction to TSWV among the genotypes tested by mechanical and thrips inoculation. Evaluation of severity of symptoms resulting from mechanical inoculation indicated that there was

significant difference among the genotypes, despite sign of systemic infection (Table 1).

The breeding lines C-11-2-29, ICGV-86388, IC-10, and IC-34 showed higher resistance than the cultivar Georgia Green, related to the intensity of symptoms and percentage of plants infected. The absence of stunting and apical death in the first four genotypes allowed continuous plant growth, which indicates potential yield, as opposed to complete yield lost caused by death among plants of the cultivars Georgia Green and SunOleic.

Georgia Green was as susceptible to TSWV as SunOleic; which is interesting, as the former is considered the standard for resistance and the later the standard for susceptibility (Culbreath et al., 2000, 2003; Brown et al., 2003). This phenomenon has been attributed to the so called field resistance of Georgia Green (Culbreath et al., 1996, 2000), in which, under experimental mechanical inoculation, no difference in susceptibility to TSWV was observed among several peanut cultivars that are less affected by disease under field conditions (Culbreath et al., 1994; Hoffmann et al., 1998). This phenomenon has been suggested to be related to plant factors that alter the insect behavior under field conditions, limiting TSWV transmission (Krishna Kumar, 1993).

More interesting was the reaction of the genotypes to TSWV by thrips inoculation. Only two genotypes (IC-34 and ICGV-86388) were infected by TSWV inoculated by thrips. Furthermore, the infection could only be detected by RT-PCR, and not by visual symptom expression or ELISA using both leaf and root tissue. ELISA generally yields an indication of TSWV in infected plants, except in cases like the one above, in which virus antigen titer at the infected plant may be below the detection level of the assay. The use of RT-PCR overcame the limitation associated with ELISA assay. In addition, to be more sensitive than ELISA, RT-PCR surveys the presence of viral genetic material.

The combination of two methods that explore different biological characteristics of the virus, permits better conclusion concerning the virus presence. When subliminal infection may occur, the use of a method with the sensitivity of RT-PCR eliminates the possibility of false negative, very frequent in ELISA assay.

The combined results from mechanical and thrips inoculations indicated that the virus inoculation method affects the genotype susceptibility to TSWV. The breeding line ICGV-86388 was one of the least susceptible by mechanical inoculation (Table 1), but was one of the two genotypes susceptible to thrips inoculation.

Hence, partial resistance may be correlated to the method of inoculation rather than a true resistance to the virus.

Previous reports indicate that the cultivar Georgia Green is not resistant to thrips infestation (Culbreath et al., 2000; Wells et al., 2002). Culbreath et al. (1992) found no resistance to thrips infestation in the cultivars Southern Runner and Florunner, yet the incidence of TSWV differed between them. They suggested that physical or physiological factors could affect the frequency of inoculation and infection processes of TSWV in the plant population.

The breeding lines IC-10, IC-34, and ICGV-86388 were derived from a germplasm line resistant to thrips (Pensuk et al., 2002). In addition, IC-10 was obtained by crossing involving a cultivar resistant to *Peanut bud necrosis virus* mediated by thrips non preference (Amin, 1985). There is the possibility that natural heterogeneity to susceptibility to thrips inoculation of TSWV exists in this breeding line. Evaluation of genotype resistance to TSWV in several crops has indicated the limitation of using only mechanical inoculation to evaluate resistance and susceptibility (Amin, 1985; Krishna Kumar et al., 1993; Culbreath et al., 1996; Wells et al., 2002).

Conclusions

1. The breeding lines IC-10, IC-34, and ICGV 86388 present higher resistance to TSWV than the standard for resistance, cultivar Georgia Green.

2. The outcome of a resistance evaluation can be linked to the inoculation method.

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