Localization of Tomato Spotted Wilt Virus (Genus *Tospovirus*, Family *Bunyaviridae*) in Peanut Pods

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ABSTRACT

The localization of tomato spotted wilt virus (TSWV, genus *Tospovirus*, family *Bunyaviridae*) in peanut pods was determined by enzyme-linked immunosorbent assay (ELISA) using TSWV specific antibodies. Pods were collected from symptomatic and asymptomatic field-grown plants. All the plants were tested by ELISA for presence or absence of TSWV infection. Normal and abnormal looking pods from symptomatic plants were assayed by ELISA. Each pod was divided into shell, testa, and cotyledons. All of the shell and testa samples of both normal and abnormal pods from symptomatic plants were positive for TSWV, whereas TSWV could not be detected in the cotyledons. Similar results were observed by polymerase chain reaction, except that the cotyledons occasionally had a TSWV-specific sequence. No virus could be detected in any part of the pod collected from asymptomatic, virus-free plants. In grow-out tests of seed from both symptomatic and asymptomatic plants, none of the plants showed TSWV infection when assayed by ELISA. Results demonstrated the preferential accumulation of the virus in the shell and testa.

Key Words: ELISA, polymerase chain reaction, seed transmission, *Tospovirus*, virus detection.

Spotted wilt, caused by tomato spotted wilt *Tospovirus* (TSWV), is an economically important disease on peanut (*Arachis hypogaea* L.), tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.), and pepper (*Capsicum annuum* Lycopersicon) in Southeastern United States. In Georgia, annual losses due to this disease are estimated at $100 million (Bertrand, 1998). First reported in Georgia in 1986, the disease has become a major constraint during the 1990s. It continues to be a yield-limiting factor in peanut in the state.

No single control strategy has been effective in reducing the impact of TSWV in peanut. However, a combination of several factors seems to affect the final disease incidence and yield in peanut (Brown *et al.*, 1997). Based on these factors, a risk index was developed for the management of TSWV in peanut in Georgia (Brown *et al.*, 1998).

TSWV is transmitted by several species of thrips. The seasonal dynamics of various thrips species colonizing peanut have been documented (Todd *et al.*, 1995, 1996). Western flower thrips [*Frankliniella occidentalis* (Pergande)] and tobacco thrips [*F. fusca* (Hinds)] are the most prevalent vector species in Georgia's peanut crop. Recently *F. bispinosa* (Morgan) was shown to transmit TSWV under experimental conditions (Webb *et al.*, 1997). Spotted wilt epidemics can be attributed largely to the prevalence of thrips vector species and the availability of virus inoculum. Thrips-mediated spread seems to be the only mode by which the virus is distributed as evidence for seed transmission is lacking (Moyer, 1999). The objective of this study was to determine the localization of TSWV in peanut pod.

Materials and Methods

In 1998, peanut plants from the plots of Coastal Plain Exp. Sta., Tifton, GA were flagged throughout the season to identify TSWV symptomatic plants. Plants were uprooted and the whole plants including the root system were individually placed in polythene bags and taken to the laboratory. Asymptomatic plants were similarly collected from the same plots.

Leaves and roots of the symptomatic plants were assayed by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit from Agdia Inc. (Elkhart, IN) following the supplier's instructions. Asymptomatic plants were similarly assayed to verify their virus-free nature. A sample was deemed positive for TSWV if the ELISA value was 3× the known uninfected sample.

Twenty each of normal and abnormal-looking pods from each group (symptomatic and healthy plants) were randomly selected and used to determine the localization of TSWV in peanut pods. Pods from symptomatic plants were individually shelled and separated into two groups—abnormal pods that showed symptoms of TSWV infection (shriveled, shrunken, or discolored seed) and those that did not have visible abnormalities ("normal" pods). Each pod was then separated into shell, testa, and cotyledons. Each subsample from a pod was then assayed by ELISA to determine the presence of TSWV.

Selected samples of shell, testa, and cotyledons were processed for immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) as described by Jain *et al.* (1998). Primers specific to the 3' end of the small RNA (Dewey *et al.*, 1996) were used. One of the primers (HRP11) was specific to the 3' terminal region of the small RNA and

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contained the sequence 5'-AGA GCA AT(C/T) GTG TCA-3'. The other primer (HRP20) was derived from the nucleocapsid gene and contained the sequence 5'-TCA AG(C/T) CTT C(G/T)G AA(A/G) GTC AT-3'. The reverse transcription was carried out at 42 C for 45 min followed by amplification for 40 cycles with the following parameters: denaturation at 94 C for 1 min, annealing at 42 C for 2 min, and extension at 72 C for 2 min (Pappu *et al.*, 1996). The final amplification cycle was followed by extension at 72 C for 10 min. The amplification products were analyzed by 0.8% agarose gel electrophoresis. Seed from both virus-infected and virus-free plants were grown in isolation, and the resulting seedlings were assayed by ELISA for the presence of TSWV.

Results and Discussion

To determine the localization of TSWV in peanut pods, several peanut plants showing symptoms of TSWV infection and several "healthy looking" asymptomatic plants were collected. Analysis of leaves and root samples by ELISA confirmed that the symptomatic plants indeed were infected with TSWV, whereas asymptomatic plants were free of TSWV.

When shell, testa, and cotyledons from each of the pods were assayed by ELISA for the presence of TSWV, 100% of the shell and testa samples that came from symptomatic plants were positive for TSWV, whereas none of the cotyledons had the virus (Table 1). Shell samples consistently gave higher ELISA values as compared to testa (not shown). TSWV was not detected in any of the shell, testa, or cotyledons collected from asymptomatic plants (Table 1). In grow-out tests, none of the plants, irrespective of the source of the seed, was positive for TSWV (data not shown). ELISA values ranged from 1.525 to 2.751 for shell samples and 1.010 to 1.51 for testa samples.

Table 1. Detection of tomato spotted wilt *Tospovirus* (TSWV) by enzyme-linked immunosorbent assay (ELISA) in peanut pod.

	Infected plants (pods)		Uninfected plants (pods)	
	Normal	Abnormal	Normal	Abnormal
Shell	20/20[a]	20/20	0/20	0/20
Testa	20/20	20/20	0/20	0/20
Cotyledons	0/20	0/20	0/20	0/20

[a]The number of ELISA positive samples/number of samples tested.

Similar results were obtained when abnormal looking pods from symptomatic plants were tested by PCR. The shell and testa were positive for TSWV-specific sequences (Fig. 1) in all cases while the cotyledons were negative in most cases. The cotyledons were occasionally found to contain TSWV-specific sequences by PCR (data not shown). This could have resulted from contamination of dissecting tools with viral RNA during tissue separation of individual seeds. In theory, a single copy of nucleic acid can be amplified to whatever level is required for detection. To rule out the possibility of carry-over during sample preparation, the three subsamples (shell, testa, and cotyledon) were processed using separate blades and the PCR was repeated. This resulted in no amplification in the cotyledon extract. The amount of the virus-specific sequences was highest in the shell compared to the testa (as judged by the intensity of the PCR product in an agarose gel) and was in agreement with the ELISA results. The preferential accumulation of TSWV in shell and testa makes them better substrates for detecting the virus.

Previous research conducted in Georgia showed no evidence of transmission through peanut seed (A.K. Culbreath and J.W. Todd, unpub. data). These studies involved collecting seed from TSWV-infected peanut plants and growing progenies. The resulting plants were then observed for TSWV-induced symptoms and were further verified by ELISA. None of the plants tested positive for TSWV, indicating that seed transmission did not occur (A.K. Culbreath and J.W. Todd, unpub. data). Our results support these findings.

Several viruses are seedborne in peanut (Sherwood and Melouk, 1995). Viruses that are seed-transmitted are usually localized in embryo (Matthews *et al.*, 1991; Maule and Wang, 1996). Viruses differ in their ability to colonize different parts of the seed (Matthews, 1991; Maule and Wang, 1996) and little is known about the effect of the intra-embryo location of the virus on its seed transmission. While spotted wilt adversely affects the peanut quality, vigor, and yield, results indicate that it is not seed-transmitted.

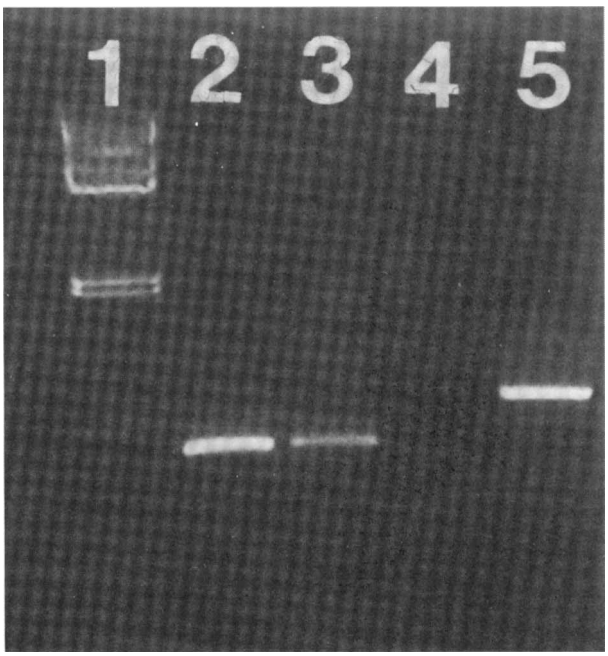

Fig. 1. Agarose gel (0.8%) electrophoresis of immunocapture-reverse transcription polymerase chain reaction (IC-RT-PCR) amplification products. Extracts from peanut shell, testa, and cotyledons taken from symptomatic peanut plants were used as templates and amplified with tospovirus-specific primers HRP11 and HRP20. Lane 1: Lambda DNA digested with *Hind*III used as a marker; lanes 2, 3, and 4: products amplified from the shell, testa, and cotyledon extracts, respectively; and lane 5: IC-RT-PCR amplification from an extract of TSWV-infected tobacco leaf used as a positive control.

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