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# Purification and some serological relationships of tomato spotted wilt virus isolates occurring on peanut (*Arachis hypogaea*) in the USA

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A procedure for the purification of TSWV-Tx, a tomato spotted wilt virus isolate infecting peanuts in Texas, is described. A rabbit antiserum was produced. Several TSWV isolates occurring on peanut in the USA reacted to varying extents in ELISA with antisera to TSWV-Tx and to Greek TSWV isolates, but failed to react with antiserum to an isolate of TSWV from India. In reciprocal tests, antigens of the Indian TSWV failed to react with antisera to Tx and to the Greek isolates. Purified TSWV-Tx contained four polypeptide species of 78 000, 58 000, 54 000 and 27 000 Da. In electro-blot immunoassays, all four polypeptides reacted with the homologous antiserum, and with antisera prepared against a Greek, a Dutch and an Australian isolate. None of the polypeptides reacted with the antiserum to the Indian isolate.

## INTRODUCTION

Halliwell & Philley (1974) first reported tomato spotted wilt virus (TSWV) on peanut (*Arachis hypogaea*) in Texas. In the early 1980s, the disease became a major constraint to peanut production in southern Texas, and it is currently considered to be one of the major peanut diseases on a global scale (Reddy *et al.*, 1983; 1991). Besides peanut, TSWV infects many vegetables and ornamentals (Reddy & Wightman, 1988; Cho *et al.*, 1989). In serological tests, a TSWV isolate from southern Texas (TSWV-Tx) failed to cross-react with an isolate from India (TSWV-Ind), which also came from peanut (D. V. R. Reddy, A. S. Ratna, M. R. Sudarshana and I. Kiran Kumar, unpublished data). We report here the purification and polypeptide composition of TSWV-Tx, and the results of comparing host range and serological relationships with several other TSWV isolates from peanut in the USA.

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## MATERIALS AND METHODS

### Virus isolates, test plants and sap transmission

Extracts from TSWV-infected peanut samples (obtained from Dr D. H. Smith, Texas A & M University), prepared in 0·05 M potassium phosphate buffer, pH 7·0, containing 0·01 M sodium sulphite, were inoculated to *Vigna unguiculata* cv. Early Ramshorn. The isolate TSWV-Tx, obtained after five successive transfers of single lesions on cowpea, was maintained in *Nicotiana tabacum* cv. Burley-21 or *Datura stramonium*. For local lesion assay, *Chenopodium amaranticolor* and *Petunia hybrida* (Ie, 1970; Boswell & Gibbs, 1983; Reddy *et al.*, 1983) were used.

TSWV isolates from Florida (TSWV-Fl) and Georgia (TSWV-Ga) were collected from peanut in 1987 (Sreenivasulu *et al.*, 1988). Each isolate, following five successive single-lesion transfers from cowpea, was propagated in *N. tabacum* cv. Burley-21.

*Heterologous antisera*

An antiserum to TSWV-Ind was obtained from the ICRISAT collection. Antisera to an Australian (TSWV-Aus), a Greek (TSWV-Grk) and a Dutch (TSWV-Nl) isolate were generous gifts from Drs D. Mossop, G. V. Gooding Jr and D. Peters, respectively.

*Virus purification*

All purification steps were followed in a cold room at *c.* 4 C. Systemically infected *N. tabacum* cv. Burley-21 or *A. hypogaea* leaves were homogenized in a Waring blender in 0·1 M potassium phosphate buffer, pH 7·0, containing 0·01 M sodium sulphite (PPBS) (3 ml/g tissue). The homogenate was squeezed through four folds of cheesecloth, and centrifuged at 5000 *g* for 15 min. Polyethylene glycol (MW 8000, Fisher Scientific Co.) and NaCl were added at 4% and 0·2 M, respectively, and after 1·5 h the precipitate was collected by centrifugation at 12000 *g* for 20 min. The pellets were suspended in PPBS (0·3 ml/g initial tissue) and clarified at 8000 *g* for 15 min. The supernatant was layered onto a 10–40% linear sucrose gradient in PPBS (prepared by adding 8·0 ml each of 10, 20, 30 and 40% w v sucrose and leaving overnight at 4 C) and centrifuged at 25000 r.p.m. for 1 h in a Beckman SW28 rotor. A light-scattering zone (4·4·5 cm from the bottom) was withdrawn, diluted in PPBS, and layered onto 25–50% linear sucrose gradients prepared as before. The tubes were centrifuged for 4 h at 25000 r.p.m. as above. A light-scattering zone (3·8–4·5 cm from the bottom) was collected and diluted in PPBS. The virus was pelleted at 35000 r.p.m. for 1·5 h in a Beckman R40 rotor.

*Antiserum production*

Purified virus (from 100 *g N. tabacum* tissue, suspended in 0·05 M potassium phosphate buffer, pH 7·0, containing 0·87% NaCl) was emulsified with an equal volume of Freund's incomplete adjuvant and injected intramuscularly into two New Zealand White inbred rabbits. Five injections were given at weekly intervals. The rabbits were bled at weekly intervals after the last injection.

*Enzyme-linked immunosorbent assay (ELISA)*

The method used was similar to the direct antigen-coating procedure of Hobbs *et al.* (1987). The TSWV isolates used were those maintained in *N. tabacum* cv. Burley-21. Crude extracts of healthy plants were prepared in 0·05 M sodium carbonate buffer, pH 9·6. The antisera were cross-adsorbed with such extracts for 45 min at 37°C (Hobbs *et al.*, 1987). Protein A–alkaline phosphatase conjugate (Sigma Chemical Co., P-9650) at 0·1 unit/ml and *p*-nitrophenylphosphate (Sigma) at 1 mg/ml were used. Plates were incubated in the dark either at room temperature (22–25°C) for 3 h, or overnight at 4°C. Results were recorded at 405 nm using a Dynatek ELISA reader.

*Electrophoresis of virus proteins*

The polypeptides from virus preparations purified from *A. hypogaea* were analysed by polyacrylamide gel electrophoresis (PAGE) in slab gels, essentially as described by Laemmli (1970). Proteins from healthy *A. hypogaea* leaves, obtained by a purification procedure similar to that used for the virus, served as controls. The concentrations of the resolving and stacking gels were 10 and 4%, respectively. Purified virus samples and marker proteins were electrophoresed at 25 V for 16 h. Phosphorylase B (97400), bovine serum albumin (66200), ovalbumin (42700), carbonic anhydrase (31000) and soybean trypsin inhibitor (21000) (Biorad) were used as molecular weight markers.

*Electro-blot immunoassay*

After PAGE, polypeptides from purified virus preparations and from healthy plants were electro-blotted to nitrocellulose paper (Schleicher and Schull BA 85, 0·45 μm pore size) and probed with different antisera as described by Naidu *et al.* (1989). Various TSWV antisera were used at 1:500 dilution, and 1-naphthyl phosphate was used as substrate. Colour development was initiated by the addition of 25 mg Fast Blue RR salt (Sigma) in 5 ml water. Results were assessed visually.

## RESULTS AND DISCUSSION

Chlorotic spots and concentric rings appeared on peanut leaflets inoculated with TSWV-Tx 2 weeks after inoculation. Fully expanded leaflets below the terminal bud later became flaccid, with necrosis of the petiole and terminal bud. This led to cessation of apical growth, and proliferation of axillary shoots bearing mottled and distorted leaflets. Symptoms on selected hosts are given in Table 1.

**Table 1.** Symptoms induced by a tomato spotted wilt virus isolate from south Texas on various host plants

| Host plant | Symptoms[a] | |
|---|---|---|
| | Local | Systemic |
| *Capsicum annuum* | CRS | CRS, M, LD |
| *Chenopodium amaranticolor* | CS or NS | -[b] |
| *Datura stramonium* | CRS | CS, M |
| *Gomphrena globosa* | CS | M |
| *Lycopersicon esculentum* | CS | M, N, B |
| *Nicotiana tabacum* cv. Burley-21 | CS or NS | CS or NS, NV, M, LD |
| *N. glutinosa* | CS or NS | N (rare) |
| *Petunia hybrida* | CS | - |
| *Phaseolus vulgaris* cv. Topcrop | CS | - |
| *Vigna unguiculata* cv. Early Ramshorn | CRS | CS (sometimes) |
| *Vigna unguiculata* cv. Clay | CRS | CS (sometimes) |
| *Vinca rosea* | BNS | M, LD |

[a] B, bronzing; BNS, black necrotic spots; CRS, chlorotic ring spots; CS, chlorotic spots; LD, leaf distortion; M, mosaic; N, necrosis; NS, necrotic spots; NV, necrosis along lateral veins. Results were confirmed by assays on *V. unguiculata*.

[b] No symptoms observed; no infective virus recovered.

**Table 2.** Detection of tomato spotted wilt virus by ELISA using unadsorbed and cross-adsorbed homologous antiserum

| Antigen (*N. tabacum*-cv. Burley-21 leaf extracts) | Dilutions of antigen[a] | Dilutions of antiserum | | | | | |
|---|---|---|---|---|---|---|---|
| | | Unadsorbed | | | Cross-adsorbed[b] | | |
| | | 1:200 | 1:1000 | 1:5000 | 1:200 | 1:1000 | 1:5000 |
| Infected | $10^{-2}$ | 1·77[c] | 1·55 | 0·67 | 1·84 | 0·69 | 0·33 |
| | $10^{-3}$ | 1·57 | 1·23 | 0·23 | 0·97 | 0·32 | 0·14 |
| Healthy | $10^{-2}$ | 1·30 | 0·90 | 0·28 | 0·09 | 0·02 | 0·03 |
| | $10^{-3}$ | 1·01 | 0·88 | 0·26 | 0·07 | 0·00 | 0·02 |

[a] Dilutions based on original weight of leaf tissue.

[b] Cross-adsorbed with healthy *N. tabacum* cv. Burley-21 leaf extracts as described by Hobbs *et al.* (1987).

[c] Mean A 405 nm value for three replicates.

Initially, many methods were tried for the purification of TSWV-Tx (Black *et al.*, 1963; Mohamed *et al.*, 1973; Tas *et al.*, 1977; Francki & Hatta, 1981). Preparations obtained by each procedure were analysed by SDS-PAGE. They contained several polypeptides, some of host origin. The method adopted is a modification of that reported for a TSWV-Ind isolate (ICRISAT, 1988). Purified virus retained approximately 5% of the infectivity present in crude plant extracts.

An antiserum produced against TSWV-Tx contained antibodies to healthy leaf extracts. However, after cross-adsorption with such extracts it could be used to detect virus in infected leaf extracts (Table 2). Weak reactions with healthy tobacco leaf extracts, observed for several TSWV antisera, could be minimized by cross adsorption with such extracts.

TSWV-Tx preparations gave high ELISA values with homologous, TSWV-NI and TSWV-

**Table 3.** Reactions of four tomato spotted wilt virus (TSWV) isolates in ELISA

| Isolates[a] | Antisera[b] | | | | |
|---|---|---|---|---|---|
| | TSWV-Tx | TSWV-Aus | TSWV-Grk | TSWV-Nl | TSWV-Ind |
| Texas | 1·80[c] | 1·66 | 0·39 | 1·44 | 0·03 |
| Georgia | 1·67 | 1·21 | 1·28 | 1·08 | 0·07 |
| Florida | 0·04 | 0·09 | 0·09 | 0·06 | 0·03 |
| India | 0·02 | 0·08 | 0·06 | 0·09 | 1·24 |

[a] Viruses maintained in *N. tabacum* cv. Burley-21, used at 1:100 dilution.
[b] Cross-adsorbed with healthy *N. tabacum* cv. Burley-21 leaf extracts.
[c] Mean A 405 nm value for three replicates.

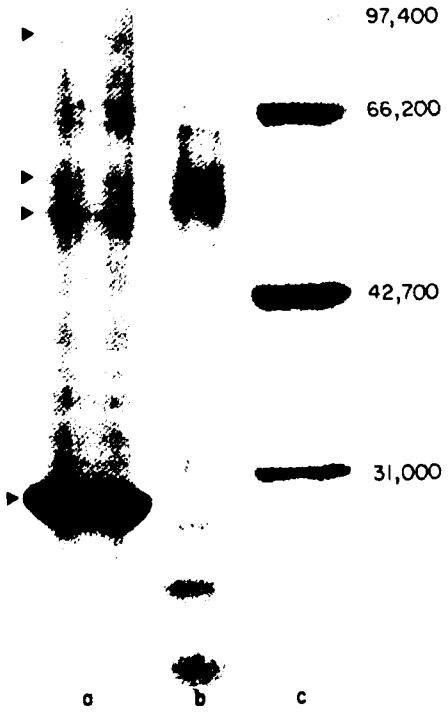


**Fig. 1.** SDS polyacrylamide slab gel showing polypeptides (arrows) of a tomato spotted wilt virus (TSWV-Tx) isolate (lane a), polypeptides from comparable but healthy peanut leaves (lane b), and molecular weight markers (lane c).

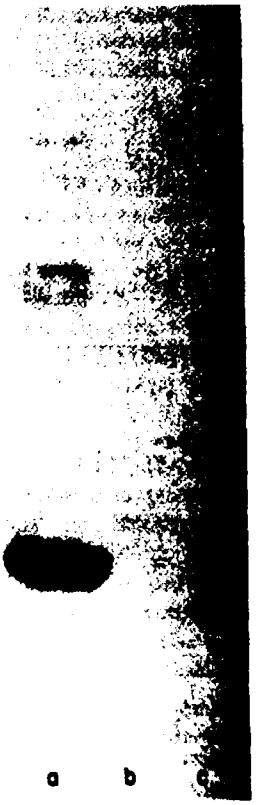


**Fig. 2.** Immuno-blots of polypeptides of a tomato spotted wilt virus (TSWV-Tx) isolate after probing with TSWV-Tx antiserum (lane a), and with antiserum for an Indian isolate (lane b). Polypeptides from comparable but healthy peanut leaf extract were probed with TSWV-Tx antiserum (lane c).

Aus antisera, but reacted weakly with antiserum to TSWV-Grk. The TSWV-Ga isolate reacted strongly with TSWV-Tx, TSWV-Grk, TSWV-Aus and TSWV-Nl antisera. TSWV-Tx and TSWV-Ga did not react with the antiserum to TSWV-Ind. TSWV-Fl did not react with any of the antisera tested (Table 3). It thus appears that

TSWV-Fl and TSWV-Ind are the only isolates not reacting with TSWV-Tx antiserum, indicating that they are serologically distinct.

Four virus-associated proteins of estimated molecular weight 78000, 58000, 54000 and 27000 Da were consistently detected by PAGE in infected but not healthy extracts (Fig. 1). Several minor virus-associated polypeptides were also observed, whose presence and concentration varied from sample to sample.

In electro-blot immunoassay, all the major proteins of TSWV-Tx could be detected with the homologous antiserum (Fig. 2) and antisera to the isolates Grk, Aus and Nl. TSWV-Ind antiserum did not react with any of the TSWV-Tx proteins, in agreement with the ELISA results. It is interesting that none of the polypeptides of TSWV-Tx reacted with TSWV-Ind antiserum. We presume that TSWV-Tx is similar to the TSWV strain isolated from lettuce (Cho *et al.*, 1989). Whether the TSWV-Fl isolate is related to the TSWV isolate from *Impatiens* (Moyer *et al.*, 1991), which is serologically distinct from the lettuce strain, is yet to be determined.

It is apparent from this study that several antisera should be used to determine serological relationships of TSWV isolates occurring in various peanut-growing regions of the world. In conducting surveys for TSWV, it would be useful to have a monoclonal antibody reacting with all virus isolates. Additionally, it is now essential to determine the host ranges of various TSWV isolates under similar conditions, so that such data can be used for diagnostic purposes.

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