Comparison of Two Strains of Peanut Stripe Virus in Taiwan

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ABSTRACT

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Two strains (Ts and Tc) of peanut stripe virus (PStV) naturally occurring in peanut (*Arachis hypogaea*) in Taiwan were isolated. PStV-Ts induced severe mosaic and systemic necrotic symptoms differing from the stripe symptoms induced by PStV-Tc and previously described PStV isolates. The biological properties of PStV-Ts were very similar to those of PStV-Tc and other known PStV isolates. Seed transmission rates of PStV-Ts in peanut averaged 12.5%. The purified capsid and cylindrical inclusion proteins of PStV-Ts consisted of single species of protein monomer with molecular weights of 36,000 and 71,000, respectively. In serological tests, PStV-Ts was indistinguishable from PStV-Tc and two PStV isolates from the United States but different from 14 other potyviruses.

Peanut, *Arachis hypogaea* L., ranks as the third most important crop in Taiwan, where about 90,000 tons are produced annually. In 1979, a virus (Ts) related to peanut stripe virus (PStV) and causing severe mosaic and necrotic and stunt symptoms on peanut, distinct from those induced by peanut mottle virus (18,19), was detected in southern Taiwan (1,2). In 1982, other isolates of this virus (Tc), which induce stripe or veinbanding but no necrosis symptoms, were also detected. This paper describes the Ts and Tc isolates from Taiwan and compares them serologically with PStV and other legume potyviruses described elsewhere.

MATERIALS AND METHODS

Source and maintenance of virus isolates. Isolates Ts and Tc were obtained from systemically infected plants of the peanut cultivar Tainung No. 4, which were inoculated from single lesions of *Chenopodium amaranticolor* Coste & Reyn. Three Tc isolates (Tc1, Tc2, and Tc3) were selected for further comparative studies with the Ts isolate and with other legume potyviruses. All these isolates were maintained either on peanut or on blackeye cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye') by manual inoculations.

Host range test. All virus isolates were transmitted manually for host range trials. At least four plants of each species were inoculated. Recovery tests were conducted by back-inoculation from test plants to *C. amaranticolor* or peanut.

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Aphid transmission tests. Aphid transmission trials were conducted with *Myzus persicae* (Sulzer). The insects were starved for 3–4 hr before access feeding. Individual aphids were given acquisition probes of approximately 60 sec on Ts-infected cowpea or Tc-infected peanut leaves. Groups of five aphids were transferred to each of 25 test plants. After a testing feeding period of 4–5 hr, the aphids were removed, and the plants were placed in a screenhouse for observation.

Electron microscopy. Virus particles in crude sap of peanut leaves inoculated with the Ts or the Tc isolate were negatively stained in 2% potassium phosphotungstate, pH 6.5, containing 0.1% (w/v) bovine serum albumin, as described elsewhere (7), and observed with a Hitachi 7000 electron microscope. The microscope was calibrated with a carbon grating replica with 2,160 lines per millimeter.

Light microscopy. Epidermal strips obtained from healthy and systemically infected cowpea leaves were treated with Triton X-100, stained either with calcomine orange and Luxol brilliant green or with azure A, and examined by light microscope for virus-induced inclusions (6).

Virus purification and antisera preparation. The purification of Ts particles and cylindrical inclusion protein of the isolate was conducted as previously described (5) from systemically infected blackeye cowpea leaves harvested 12–14 days after inoculation. Before immunizations, purified virus particles were either untreated (nondegraded) (16) or treated (degraded) as follows. About 2–3 mg of purified virus was dissociated in 1% (w/v) sodium dodecyl sulfate (SDS) in a water bath at 80 C for 2 min, dialyzed against 0.1% SDS at 4 C for 12 hr, and used in one dose. The immunization was done as described by Purcifull and Batchelor (16). For antiserum against nondegraded Ts, three injections were administered, each with 1.5 mg of intact purified Ts virus. For antiserum against degraded Ts, four injections were given, each at a similar dosage. Antisera collected 1–3 mo after the last injection were used in this study.

Serological tests. SDS immunodiffusion tests were done in a medium containing 0.8% noble agar, 0.5% SDS, and 1.0% sodium azide (16). The reactants were arranged so that homologous antigens were placed in wells adjacent to the heterologous ones for comparison. SDS-treated antigens and antisera of the following potyviruses were used: PStV isolates from Georgia (11) and Florida (19), peanut mottle (19), two isolates of bean common mosaic (13), blackeye cowpea mosaic (3), clover yellow vein (6), the PV-2 (6) and B2 (14) isolates of bean yellow mosaic, soybean mosaic (C. A. Chang, *unpublished report*), papaya ringspot W (15), watermelon mosaic 2 (15), zucchini yellow mosaic (15), potato Y (16), passionfruit woodiness (4), passionfruit mottle (4), pepper mottle (16), and tobacco etch (16).

Direct (8) and indirect enzyme-linked immunosorbent assays (ELISA) (6) were used to study relationships between Ts, PStV, and other potyviruses. Pea seedborne mosaic virus, not tested in immunodiffusion tests, was included in these trials. Each virus was propagated for 10–14 days on its host plant before it was used as an antigen in ELISA tests.

Polyacrylamide gel electrophoresis of viral and inclusion proteins. The purified capsid and cylindrical inclusion proteins were analyzed by discontinuous SDS polyacrylamide gel electrophoresis as previously described (6).

Determination of seed transmission percentage. Fifty plants of the peanut cultivar Tainung No. 4 were inoculated at the four-leaf stage, and their seeds harvested 100 days later were used to assess the seed transmissibility of Ts. Seeds were germinated on a sandbench, and the number of seedlings with Ts symptoms was recorded. Plants without symptoms were assayed in direct ELISA tests for Ts.

RESULTS

Host range and symptomatology. Forty-four species in eight plant families were inoculated with either Ts or Tc1.

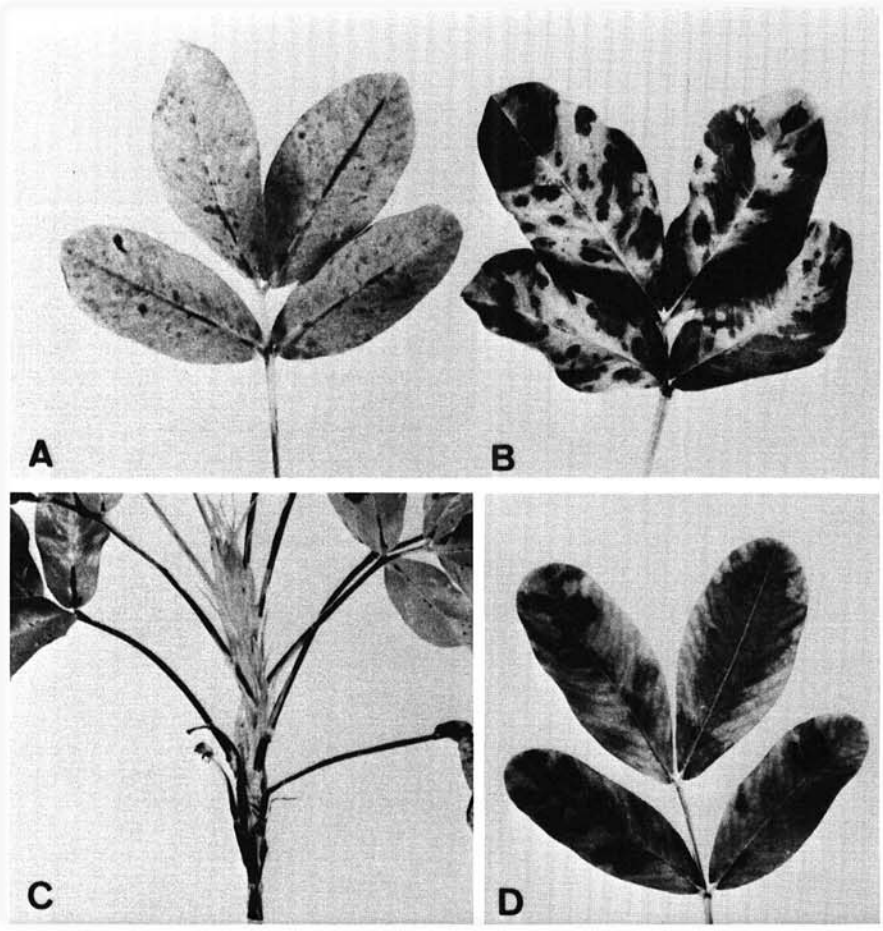

Fig. 1. Reactions of the peanut cultivar Tainung No. 4 to inoculation with the Ts and Tc isolates of peanut stripe virus in Taiwan. **(A)** Local necrosis on the midribs, petiole, and lower surface of a leaf about 20 days after inoculation with Ts. **(B)** Severe systemic mosaic caused by Ts about 14 days after inoculation. **(C)** Systemic necrosis induced by Ts on petioles and stem about 1 mo after inoculation. **(D)** Systemic stripe symptoms caused by Tc.

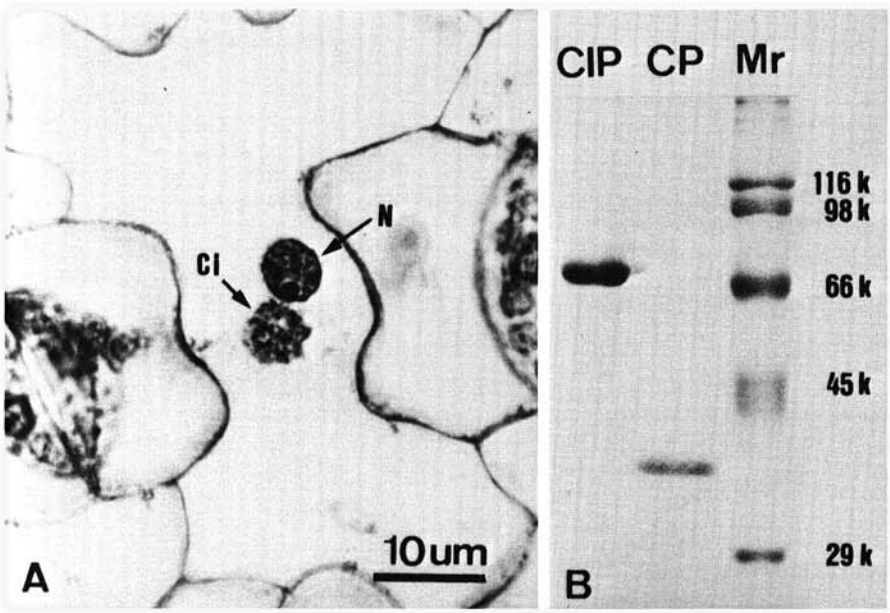

Fig. 2. (A) Light micrograph of epidermal tissue of blackeye cowpea 15 days after inoculation with the Ts isolate, which was processed with Luxol brilliant green and calcomine orange stains, showing a cylindrical inclusion (CI) in cytoplasm beside a nucleus (N). **(B)** Photomicrograph of purified CI protein (CIP) and capsid protein (CP) as analyzed in sodium dodecyl sulfate polyacrylamide gel electrophoresis, with molecular weight marker proteins (Mr).

Chlorotic lesions appeared on systemically infected leaves of four cultivars of *A. hypogaea* (Tainung No. 4, Tainung No. 5, Tainan Selection No. 9, and Tainan No. 11) about 7–20 days after inoculation with Ts. Spots on the lower leaf surface gradually became necrotic, and the necrosis extended to the midribs and petioles and sometimes to the stems (Fig. 1A and C). At a later stage, plants infected with Ts were stunted and subsequently developed severe mosaic, systemic foliar distortion, or stripe symptoms (Fig. 1B). In contrast, necrosis, stunt, and chlorotic lesions were not observed when *A. hypogaea* was inoculated with Tc1. Tc-infected plants developed only the stripe symptom (Fig. 1D), closely resembling symptoms of PStV infection described in the United States (11).

In addition to *A. hypogaea*, the following plants were systemically infected by both viruses and expressed foliar mosaic symptoms: *Nicotiana benthamiana* Domin, *V. unguiculata* subsp. *unguiculata* 'California Blackeye,' *V. angularis* (Willd.) Ohwi & Ohashi, *V. mungo* (L.) Hepper, *V. umbellata* (Thunb.) Ohwi & Ohashi, and *Glycine max* (L.) Merr. *V. unguiculata* subsp. *sesquipedalis* (L.) Verdc. became infected by Ts and developed foliar systemic mottle symptoms but were not infected by Tc1.

The following species developed local lesions on inoculated leaves, and the viruses were not recovered from uninoculated symptomless ones: *V. radiata* (L.) R. Wilczek and *C. amaranticolor*. On *C. quinoa* Willd., Ts induced both local lesions and systemic mosaic symptoms, whereas Tc1 induced only local lesions.

The following species showed no symptoms, and the viruses were not recovered from them: *Amaranthus mangostanus* L., *Antirrhinum majus* L., *Brassica juncea* (L.) Czernj. & Cosson, *Cassia occidentalis* L., *Chrysanthemum* × *morifolium* Ramat., *Citrullus vulgaris* Schrad., *Cucumis melo* L., *C. sativus* L., *Datura stramonium* L., *Gomphrena globosa* L., *Lagenaria siceraria* (Molina) Standl., *Lycopersicon esculentum* Mill., *Momordica charantia* L., *Nicotiana debneyi* Domin, *N. repanda* Willd., *N. rustica* L., *N. sylvestris* Speg. & Comes, *N. tabacum* L., *Petunia* × *hybrida* Vilm., *Pisum sativum* L., *Raphanus sativus* L., and *Vicia faba* L.

Aphid transmission trials. Twenty peanut seedlings were used to test the aphid transmissibility of Ts and Tc1; 18 and 15 seedlings, respectively, became infected.

Electron microscopy. Flexuous, rod-shaped virus particles were observed in negatively stained peanut extracts infected with Ts and Tc. Numerous flexuous rods were also observed in purified preparations of Ts. A total of 121 Ts particles and 107 Tc particles were chosen for examination. The mean lengths

were 760 and 747 nm, respectively.

Light microscopy. Cytoplasmic cylindrical inclusions were consistently observed in cells of epidermal strips of Ts- and Tc-infected cowpea and peanut leaves (Fig. 2). No nuclear or amorphous-like inclusions were observed in infected tissues stained either with calcomine orange and brilliant green or with azure A.

Serology. Antiserum to nondegraded Ts reacted strongly with its homologous antigens in ELISA but only weakly in SDS immunodiffusion tests. Antiserum to degraded Ts, however, reacted strongly with its homologous antigen in SDS immunodiffusion tests. The respective antiserum titers of degraded and nondegraded Ts were 1/4 and 1/16 in the SDS immunodiffusion tests. Neither antiserum reacted with antigens from healthy plant tissue. In reciprocal SDS immunodiffusion tests, antiserum to degraded Ts did not react with antigens of bean yellow, clover yellow vein, papaya ringspot type W, passionfruit mottle, passionfruit woodiness, peanut mottle, pepper mottle, potato Y, tobacco etch, and zucchini yellow mosaic viruses. However, strong reactions, without spurs, were noted between Ts antiserum and antigens of Tc1, Tc2, Tc3, and the PStV isolates from Georgia and Florida (Fig. 3). Ts antiserum also reacted, with spur reactions, against antigens of bean common mosaic, blackeye cowpea mosaic, soybean mosaic, and watermelon mosaic 2 viruses. Reciprocally, spur reactions were observed between antisera against bean common mosaic, blackeye cowpea mosaic, soybean mosaic, and watermelon mosaic virus 2 and antigens of Ts, Tc, and the PStV isolates from Florida and Georgia (Fig. 3 and Table 1).

In direct and indirect ELISA, Ts and Tc reacted closely to each other and to blackeye cowpea mosaic virus, but not to six other potyviruses tested (Table 2). In contrast to the result obtained in the SDS immunodiffusion test, neither isolate of bean common mosaic virus reacted against antiserum of nondegraded Ts. In indirect ELISA, antiserum to nondegraded Ts reacted weakly with soybean mosaic virus. The absorbance values of homologous and heterologous antigens were similar in indirect ELISA, whereas in direct ELISA the absorbance values of homologous antigens were always higher than those of heterologous antigens (Table 2).

Polyacrylamide gel electrophoresis of viral proteins. Purified Ts virus and cytoplasmic cylindrical inclusion proteins analyzed in 12% or a 7.5–15% gradient of polyacrylamide gels contained single species of protein monomer with molecular weights of 36,000 and 71,000, respectively (Fig. 2).

Seed transmission. Of 400 seeds harvested from Ts-infected peanut plants, 12.5% were infected. The study involved four replicates consisting of 100 seeds each; the number of infected seedlings from each replicate was five, nine, 25, and 11. The average germination rate of the seeds from infected plants was 81.53%, compared to 93.3% for seeds from noninfected plants.

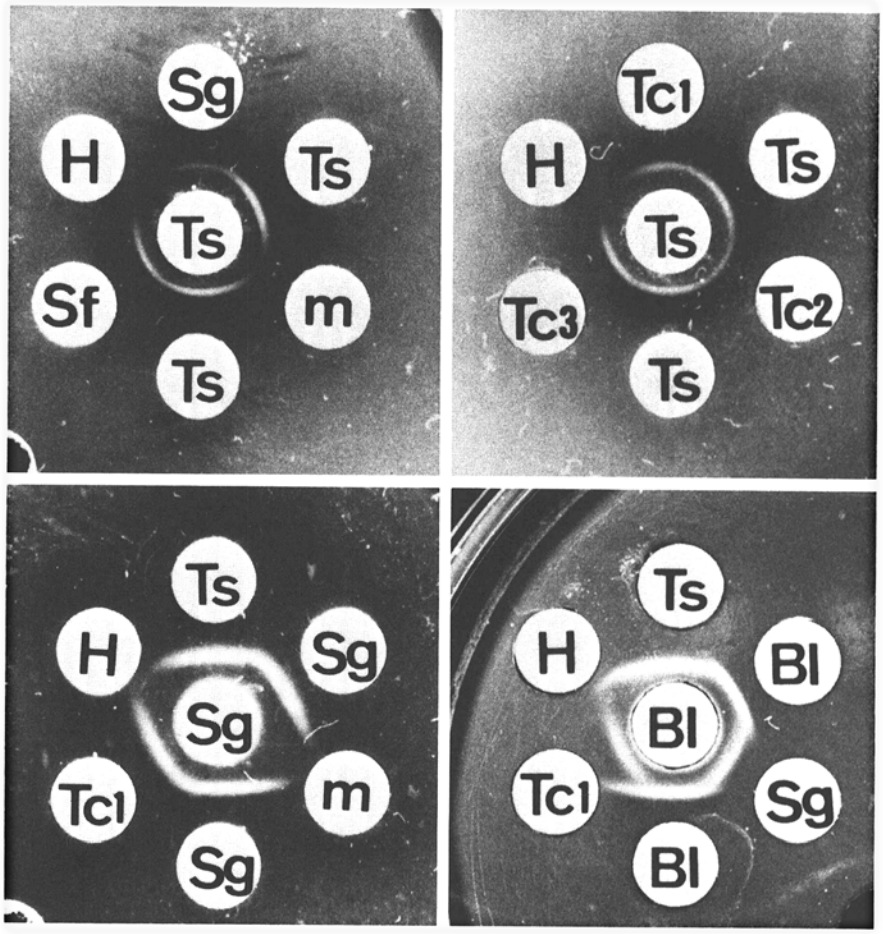

Fig. 3. Sodium dodecyl sulfate (SDS) immunodiffusion tests with the Ts isolate and three Tc isolates of peanut stripe virus (PStV) from Taiwan, two PStV isolates from the United States, blackeye cowpea mosaic virus (BlCMV), and peanut mottle virus (PMoV). SDS-treated extracts of virus-infected tissue were used as antigens to react with undiluted antiserum. The center wells were charged with antiserum against Ts, a PStV isolate from Georgia (Sg), and BlCMV (Bl). The peripheral wells were filled with antigens from Ts-infected peanut (Ts), peanut infected with the PStV isolate from Georgia (Sg), peanut infected with a PStV isolate from Florida (Sf), peanut infected with three Tc isolates from Taiwan (Tc1, Tc2, and Tc3), BlCMV-infected cowpea (Bl), PMoV-infected peanut (m), and healthy peanut (H).

Table 1. Summary of antigenic relationships between isolates of peanut stripe virus and some legume viruses as determined by sodium dodecyl sulfate (SDS) immunodiffusion tests[a]

	Cross-reactivities to SDS-treated antigens[b,c]								
	PStV				BlCMV		BCMV		
Antiserum	Ts	Tc	G	F	T	F	US1	WMV-2	SMV
PStV-Ts	I	I	I	I	S	S	S	S	S
PStV-G	I	I	I	I	S	S	S	S	S
BlCMV-F	S	S	S	S	I	I	S	S	S
BlCMV-T	S	S	S	S	I	I	S	S	S
BCMV-US1	S	S	S	S	S	S	I	S	S
WMV-2	S	S	S	S	S	S	S	I	S
SMV	S	S	S	S	S	S	...[d]	S	I

[a] SDS immunodiffusion tests were done as described by Purcifull and Batchelor (16).
[b] BCMV = bean common mosaic virus (isolate US1); BlCMV = blackeye cowpea mosaic virus (isolate F, from Florida, and isolate T, from Taiwan); PStV = peanut stripe virus (isolates Ts and Tc, from Taiwan; isolate F, from Florida; and isolate G, from Georgia); SMV = soybean mosaic virus; WMV-2 = watermelon mosaic virus 2.
[c] Results of cross-reactivities are the reactions between homologous and heterologous antigens: I = precipitin lines of homologous and heterologous reactions fused, without spur formation; S = homologous reactions spurred over heterologous reactions.
[d] No reaction occurred.

Table 2. Antigenic cross-reactivities of eight different potyviruses to antiserum against the Ts isolate of peanut stripe virus in direct and indirect enzyme-linked immunosorbent assays (ELISA)[a]

	Cross-reactivities[c]					
	Dilution of coating antigen in indirect ELISA			Dilution of antigen in direct ELISA		
Antigen[b]	5^{-1}	5^{-2}	5^{-3}	5^{-1}	5^{-2}	5^{-3}
PStV-Ts	6.24	8.96	10.67	6.61	16.18	13.22
PStV-Tc1	7.58	10.89	8.36	5.45	8.66	7.59
PStV-Tc2	6.10	8.18	8.16	4.53	4.09	4.07
BlCMV	5.02	6.50	7.92	4.01	6.32	5.23
BYMV-B2	1.02	1.35	1.56	0.13	0.51	0.48
BYMV-G4	1.00	1.17	1.54	0.34	0.99	0.87
PSBMV	0.96	0.92	0.86	0.28	0.56	0.77
SMV	1.01	3.06	3.00	0.41	1.14	0.94
BCMV-US1	0.41	0.14	0.43	0.54	1.22	0.72
BCMV-NY15	0.37	0.63	0.82	0.44	0.92	0.79
PWV	0.67	0.88	0.36	0.54	0.66	0.78
PaMV	0.77	0.98	0.58	0.66	0.72	0.83

[a] Direct and indirect ELISA were conducted as described in the text. Immunoglobulin G (IgG), purified from antiserum to nondegraded Ts collected 1 mo after the last immunization, was used at a concentration of 1 μg/ml. The IgG conjugated with alkaline phosphatase was used at a dilution of 1/1,000. Goat antirabbit IgG alkaline phosphatase conjugate diluted 1/1,000 was used in indirect ELISA tests. Virus-infected tissues were ground in a buffer solution at a ratio of 5 ml/g. The sap obtained after filtration through cheesecloth was at a 5^{-1} dilution.

[b] BCMV = bean common mosaic virus (isolates US1 and NY15); BlCMV = blackeye cowpea mosaic virus (isolate from Taiwan); BYMV = bean yellow mosaic virus (isolates B2 and G4); PaMV = passionfruit mottle virus; PStV = peanut stripe virus (isolates Ts, Tc1, and Tc2, from Taiwan); PSBMV = pea seedborne mosaic virus; PWV = passionfruit woodiness virus; SMV = soybean mosaic virus.

[c] Cross-reactivity is indicated as the ratio between the absorbance value (at 405 nm) of a virus-infected sample and that of a healthy control sample. Viruses with values greater than 2 were considered serologically related to isolate Ts.

DISCUSSION

The serological results and other characteristics of Ts and Tc shown in this study indicate that these isolates found in Taiwan are apparently strains of PStV (12). However, Ts differs from other isolates of this virus in the severity of the symptoms it induces in peanut cultivars. Also, unlike Tc isolates, Ts induced systemic symptoms in *C. quinoa* and *V. unguiculata* subsp. *sesquipedalis*. Ts and Tc were not distinguished cytologically or in reciprocal serological tests with polyclonal antisera. Similar differences in symptoms between strains of PStV have been described by others (10,12,17), but this is the first report of a strain causing such severe, necrotic symptoms in peanut. Such a strain is likely to have a much more serious affect on peanut yields than the ones described previously from Taiwan and elsewhere (10,12). In studies with Ts-infected plants grown under controlled screenhouse conditions, yield losses of as much as 67% have been recorded for the cultivar Tainung No. 4 (C. A. Chang, *unpublished data*). Moreover, if this strain is introduced to a new location through contaminated seeds, it could be readily transmitted to healthy plants by aphids.

When Ts was first described in Taiwan, it was referred to as peanut mosaic virus (1,2). Even though those reports predate the naming of PStV, the latter name should be substituted for peanut mosaic, as proposed by Demski et al (12) for peanut mild mottle and other presumed synonyms of PStV.

Ts is seedborne and thus can be shipped to other geographical areas. The apparent inability of polyclonal antisera to distinguish Ts from less severe strains of PStV precludes the use of this method for intercepting it in contaminated seed lots. Monoclonal antibodies, however, provide a potential mechanism for overcoming this problem. Culver and Sherwood (9) recently obtained a PStV monoclonal antibody for detecting this virus in peanut seed. However, its use in distinguishing different PStV strains was not considered. Of course, a monoclonal antibody with a high degree of strain specificity might not detect severe isolates, such as Ts.

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