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Peanut yellow spot virus: A distinct tospovirus species based on serology and nucleic acid hybridisation

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Summary

Nucleocapsids of peanut yellow spot virus (PYSV), purified from peanut (= groundnut) plant tissue, contained a protein with a molecular mass of 29 kDa. In ELISA and immuno-blot analysis the virus did not react with tomato spotted wilt virus (TSWV), Impatiens necrotic spot virus (INSV) and peanut bud necrosis virus (PBNV) antisera. PYSV contained three RNA species, a large (L) RNA (*c.*8900 nucleotides), a medium (M) RNA (*c.*4800 nucleotides) and a small (S) RNA (*c.*3000 nucleotides), similar to other tospoviruses. In addition, a fourth RNA species of approximately 1800 nucleotides was also present in purified preparations. Hybridisation analysis under high stringency conditions revealed no hybridisation between PYSV RNAs and cDNA probes representing the nucleocapsid (N) gene, the glycoprotein (GP) gene and the 3′ half of the RNA polymerase gene of PBNV. PYSV genomic RNAs also failed to hybridise with cDNA probes from the GP genes of TSWV and INSV. In reciprocal tests, the cDNA clones of PYSV S and M RNAs did not hybridise with any of the PBNV RNAs. Based on the absence of serological relationships between PYSV and PBNV, TSWV and INSV and lack of nucleotide homology based on hybridisation studies between the PYSV RNAs and cDNA clones from PBNV, TSWV and INSV, PYSV should be considered as a distinct species of the genus *Tospovirus* under a new serogroup, putatively designated 'V'.

Key words: Peanut yellow spot virus, tospovirus, nucleocapsids, ELISA, immuno-blot, nucleic acid hybridisation

Introduction

Viruses included in the family *Bunyaviridae* are characterised by quasi-spherical enveloped particles containing three linear ssRNA species, denoted small (S) RNA, medium (M) RNA and large (L) RNA (Elliott, 1990). They infect animals (genera *Bunyavirus, Hantavirus,*

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Nairovirus and *Phlebovirus*), and those that infect plants are classified in to a separate genus, *Tospovirus,* named after the type species tomato spotted wilt virus (TSWV) (Murphy *et al.*, 1995). Tospoviruses are transmitted by thrips species and replicate in their insect vector (Wijkamp *et al.*, 1993; Ullman *et al.*, 1993). The three genomic RNAs of tospoviruses form pseudocircular structures associated with a nucleocapsid (N) protein and few copies of a large protein (L protein), the putative viral RNA polymerase. Two glycoproteins, possibly required for thrips transmission, with molecular masses of *c.* 78 kDa (G1) and *c.* 58 kDa (G2) are embedded in the viral envelope (Mohamed, Randles & Francki, 1973; Tas, Boerjan & Peters, 1977).

The genus *Tospovirus* consists of at least four highly diverged groups, differentiated on the basis of serological cross-reactions (Adam, Yeh, Reddy & Green, 1993; de Avila *et al.*, 1993*a,b*) and amino acid sequence homology of the N proteins (de Haan, Wagemakers, Peters & Goldbach, 1990; Law, Speck & Moyer, 1991; de Avila *et al*, 1993*a*; Heinze, Maiss, Adam & Casper, 1995; Satyanarayana *et al.*, 1996*a*; Yeh & Chang, 1995). Serogroup I is comprised of TSWV isolates (de Haan *et al.*, 1990; Maiss, Ivanova, Breyel & Adam, 1991; Pang *et al.*, 1994) that react weakly with antibodies to serogroup II [TSWV-B; tomato chlorotic spot virus (TCSV); groundnut ring spot virus (GRSV)] (de Avila *et al.*, 1993*a*; Pang, Slightom & Gonsalves, 1993) and do not react with antibodies to serogroup III [Impatiens necrotic spot virus isolates (INSV)] (de Haan *et al.*, 1992; Law *et al.*, 1991; Pang *et al.*, 1994). Members of the serogroup IV, peanut bud necrosis virus (PBNV) and watermelon silver mottle virus (WSMV) (Heinze *et al.*, 1995; Satyanarayana *et al.*, 1996*a,b*; Yeh & Chang, 1995) are serologically distinct from the members in serogroups I, II and III (Adam *et al.*, 1993). Serogroup I and II members show 77–80% sequence homology between their N proteins, while serogroup I shows 55% and 33–36% sequence homology with the N proteins of serogroups III and IV, respectively.

Peanut yellow spot virus (PYSV), which in peanut leaves causes yellow spots which later coalesce and become necrotic, was reported as a distinct tospovirus based on the particle morphology, host range and serology with TSWV and PBNV antisera (Reddy *et al.*, 1991). The incidence of PYSV in farmers' fields can reach up to 90% although yield losses due to this virus have not been determined. Here we report the purification of PYSV nucleocapsids, serological relationships with tospoviruses in serogroups I, III and IV, and molecular mass estimation of the nucleocapsid protein. We also report results of Northern-hybridisation analysis of the three PYSV genomic RNAs with cDNA probes of the nucleocapsid (N) gene of PBNV, the glycoprotein precursor (GP) genes of PBNV, TSWV and INSV and the 3′ half of the RNA polymerase gene of PBNV.

Materials and Methods

Virus isolate

Peanut (= groundnut, *Arachis hypogaea* L.) plants exhibiting typical yellow spot symptoms of PYSV were collected from field plots at the ICRISAT-Asia Center, India. The virus was isolated and further maintained as described by Reddy *et al.* (1991).

Purification of nucleocapsids

PYSV nucleocapsids were purified from frozen (−70°C) leaf tissue of infected quadrifoliates as described by Satyanarayana *et al.* (1996*a*) for PBNV nucleocapsids. The procedure consisted of homogenising leaf tissue in chilled 0.1 M potassium phosphate buffer,

pH 7.6 containing 0.01 M sodium sulfite, and 0.01 M EDTA (pH 8.0) (4ml g^{-1} tissue). After clarification (800 ***g*** for 10 min) the extract was subjected to high speed centrifugation (50 000 ***g*** for 40 min) and pellets suspended in a buffer containing 10 mM each of potassium phosphate (pH 7.6), sodium sulfite and EDTA (pH 8.0) (PSE) and 1.0% Nonidet P-40 (Shell Chemicals). The resuspended pellets were stirred for 30 min, clarified at 6000 ***g*** for 10 min and the supernatant was centrifuged at 100 000 ***g*** for 75 min through a 30% sucrose cushion (15 ml) in PSE buffer. The pellet was suspended in PSE buffer and centrifuged at 140 000 ***g*** for 2.5 h (in a Beckman SW 41 rotor) in 20–40% linear sucrose gradients (3 ml of 40%, 2 ml each of 35%, 30%, 25% and 20%) prepared in PSE buffer. The three distinct light scattering zones were collected separately and nucleocapsids were concentrated by pelleting at 100 000 ***g*** for 2 h. Preparations of purified nucleocapsids suspended in 0.01 M potassium phosphate buffer, pH 7.0, were scanned in Shimadzu UV-Visible recording spectrophotometer from 200–300 nm.

Polyacrylamide gel electrophoresis of nucleocapsid protein

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of purified nucleocapsid preparations was done according to the procedure of Laemmli (1970), as described by Reddy *et al.* (1991). Purified PBNV nucleocapsid protein was included as a control. Gels were stained with 0.2% Coomassie Brilliant Blue R 250. Albumin (bovine) (66 000), albumin (egg) (45 000), glyceraldehyde-3-phosphate dehydrogenase (36 000), carbonic anhydrase (29 000), trypsinogen (24 000), trypsin inhibitor (20 100) and lactalbumin (14 200) (Sigma Chemicals) were used as molecular mass markers.

Serological analysis

Direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA) (Hobbs, Reddy, Rajeshwari & Reddy, 1987) and immuno-blot analysis (Reddy *et al.*, 1991) were used to determine serological relationships. All the antisera (cross-adsorbed with virus-free groundnut leaf extracts) were used at 1:1000 dilution for DAC-ELISA and at 1:500 dilution for immunoblots.

Extraction and analysis of RNA

The nucleocapsids from the top, middle and bottom zones of the sucrose gradient were suspended in 10 mM potassium phosphate buffer, pH 8.0, lysed [at 65°C for 15 min in 1.0% SDS and 20 mM EDTA, (pH 8.0)] and extracted with phenol, followed by phenol:chloroform:isoamyl alcohol (25:24:1, v/v) and chloroform:isoamyl alcohol (24:1, v/v). The RNA was precipitated at −20°C overnight after adding 2.5 volumes of ethanol in the presence of 300 mM sodium acetate, pH 5.2. The RNA was pelleted, suspended in diethyl pyrocarbonate (DEPC) treated water and stored at −70°C. It was analysed on agarose gels (1.0%, w/v) containing 10 mM methyl mercury (Bailey & Davidson, 1976).

Molecular cloning of PYSV S and M RNAs

The S and M RNAs of PYSV, following separation in denatured agarose gels (Bailey & Davidson, 1976), were gel eluted according to Sambrook, Fritsch & Maniatis (1989). Random primed first- and second-strand synthesis was performed as described by Gubler & Hoffman (1983) using the SuperScript Choice System (Gibco BRL) and cloned into pGEM-7Zf (+) (Promega). RNA specificity of recombinant clones was determined by Northern blot analysis and two cDNA clones belonging to S RNA (1100 bp) and M RNA (750 bp) of PYSV were used to probe Northern blots of PYSV and PBNV RNAs.

Northern blot hybridisation of PYSV RNAs

The gel-separated RNA species were transferred on to a nylon membrane by capillary transfer (Sambrook *et al.,* 1989). The tospovirus cDNA clones used to probe Northern blots were from: the S and M RNAs cDNA clones of PYSV described above; the N, GP and the 3′ half of the RNA polymerase genes of PBNV (Satyanarayana *et al.*, 1996*a,b*; S Gowda *et al.*, unpublished); and the GP genes of TSWV and INSV (Kormelink *et al.*, 1992; Law, Speck & Moyer, 1992). The cDNA inserts were gel eluted and random prime labelled with ^{32}P as described by the manufacturer (Promega). Prehybridisation (3 h) and hybridisations (16 h) were carried out at 65°C in 0.5 M sodium phosphate buffer, pH 7.2 containing 7.0% SDS and 1 mM EDTA (pH 8.0). The blots were washed with 1 × SSC containing 0.1% SDS twice for 15 min each at room temperature, followed by 0.1 × SSC containing 0.1% SDS twice for 30 min each at 65°C. The blots were dried and exposed to X-ray film.

Results

Purification of nucleocapsids and spectral properties

The nucleocapsids were separated following sucrose gradient centrifugation into three light-scattering zones, top, middle and bottom, between 6.2–6.5, 5.7–6.0 and 5.0–5.4 cm, respectively from the bottom of the tube. The UV absorption spectrum of nucleocapsids from the three zones showed the maximum and minimum absorption at 260 nm and 249 nm, respectively. The 260/280 ratio of nucleocapsids was 1.37, indicating that the nucleocapsids contained about 12% nucleic acid (Gibbs & Harrison, 1976).

Nucleocapsid protein

The nucleocapsids from all the three zones contained a single polypeptide with a molecular mass of 29 kDa (average of three determinations) (Fig.1, lane a). Differences in the apparent molecular mass of the nucleocapsid proteins between the PYSV and PBNV could be resolved (Fig. 1, lane c).

Serological relationships

In DAC-ELISA, PYSV reacted strongly with the homologous antiserum (Table 1). The PYSV antigen did not react with PBNV (Serogroup IV), TSWV (serogroup I) or INSV (serogroup III) antisera. In immuno-blots, PYSV nucleocapsid protein did not react with antisera to TSWV, INSV or PBNV, and only reacted with the homologous antiserum (data not shown).

Analysis of viral RNA

Total RNA from purified nucleocapsids was fractionated under denaturing conditions into three distinct species with sizes similar to those of PBNV (Fig. 2). The top zone contained the small (S) RNA and detectable levels of the medium (M) RNA, the middle zone contained the M, S and large (L) RNAs in decreasing concentrations, whereas the bottom zone contained the L, M and S RNAs in approximately equimolar concentrations. The size of L, M and S RNA species was estimated to be 8900, 4800 and 3000 nucleotides, respectively, similar to PBNV RNAs (Fig. 2). All three zones contained a small RNA species of 1800 nucleotides in length.

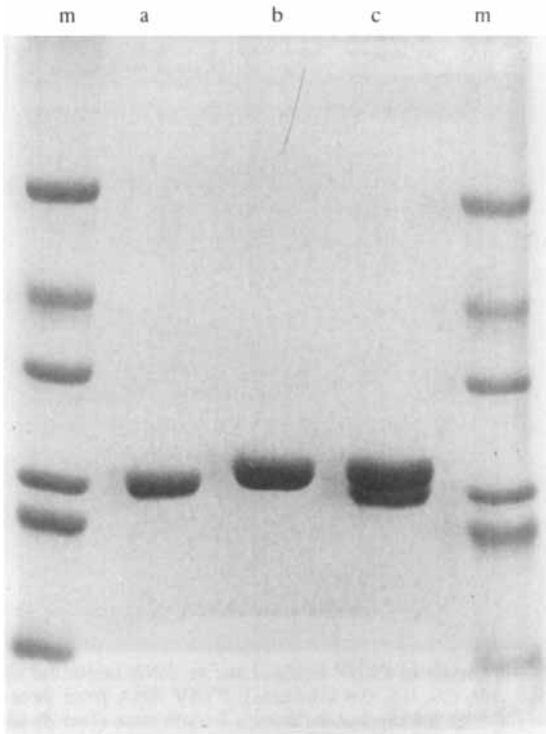

Fig. 1. SDS-polyacrylamide gel (12%) electrophoresis of PYSV and PBNV nucleocapsids. Lane m: Protein molecular mass markers (from top to bottom 66 000; 45 000; 36 000; 29 000; 24 000; 20 100 and 14 200 kDa), lane a: PYSV nucleocapsids from three zones, lane b: PBNV nucleocapsids and lane c: co-migration of PYSV and PBNV nucleocapsids.

Northern blot analysis of PYSV RNA

The PYSV RNAs did not hybridise with ^{32}P-labelled cDNA probes representing the N, GP or the 3′ half of the RNA polymerase genes of PBNV (Fig. 3), nor did they hybridise with cDNA probes from the GP genes of TSWV and INSV (data not shown). The cDNA clones of PYSV S and M RNAs hybridised specifically with the S and M RNAs of PYSV but failed to

Table 1. *Serological relationship of peanut yellow spot virus (PYSV) in DAC-ELISA*[a]

Antigen[b]	Antisera to[c]			
	PYSV	PBNV	TSWV	INSV
PYSV	1.10	0.01	0.03	0.08
PBNV	0.04	2.01	0.06	0.09
TSWV	0.07	0.03	0.62	0.10
Healthy	0.06	0.05	0.09	0.07

[a]Data given are A_{405} values of samples after deducting the signal given by control samples in which buffer alone was used instead of sample preparation. Each value is an average of three replicate wells. The experiment was made twice,

[b]Antigen at 1:10 dilution.

[c]Antisera at 1:1000 dilution.

PBNV: Peanut bud necrosis virus; TSWV: Tomato spotted wilt virus; INSV: Impatiens necrotic spot virus.

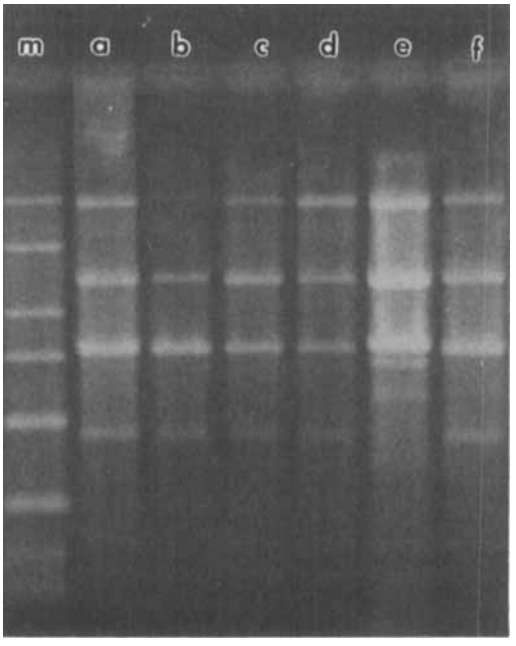

Fig. 2. Agarose gel electrophoresis of PYSV RNAs. Lane m: RNA molecular size markers (from top to bottom 9.5, 6.2, 3.9, 2.8, 1.9, 0.9, 0.6, 0.4 kilobases), PYSV RNA from three sucrose gradient zones (lanes a & f), top zone (lane b), middle zones (lane c), bottom zone (lane d) and PBNV RNA (lane e).

hybridise with the L, M or S RNAs of PBNV (Fig. 3). In addition, the PYSV S RNA cDNA clone hybridised with total nucleic acids from PYSV infected tissue and not with total nucleic acids from PBNV infected tissue in nucleic acid spot hybridisation (Fig. 4). As expected, ^{32}P-labelled cDNA probes prepared by random priming of PYSV RNAs, hybridised with PYSV and not with PBNV RNAs (data not shown).

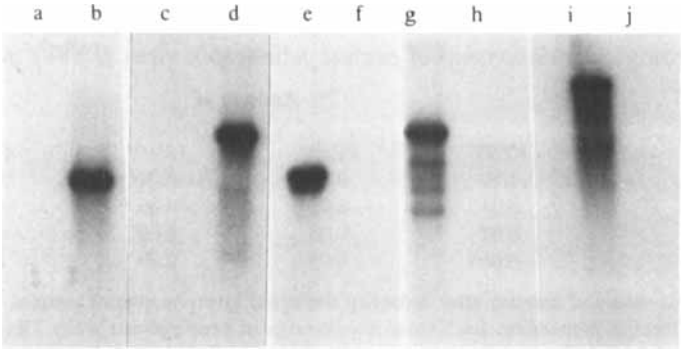

Fig. 3. Northern blot hybridisation of PBNV (lanes a, c, e, g and i) and PYSV (lanes b, d, f, h and j) RNAs using ^{32}P-labelled cDNA probes specific to PYSV S RNA (a and b), PYSV M RNA (c and d), PBNV nucleocapsid gene (e and f), PBNV glycoprotein precursor gene (g and h) and the 3′ half of the *RNA* polymerase gene of PBNV (i and j).

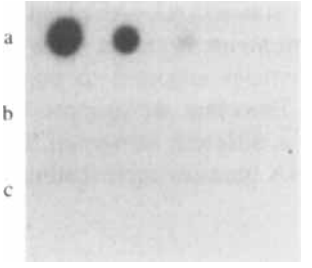

Fig. 4. Nucleic acid spot hybridisation of total nucleic acids (10-fold dilutions) from PYSV infected (a), PBNV infected (b) and virus-free (c) peanut leaf tissue using cDNA clone specific to PYSV S RNA.

Discussion

PYSV shares several phenotypic characters with other members of genus *Tospovirus*. These include particle morphology, thrips transmission (Reddy *et al.*, 1991), the presence of three genomic RNAs and nucleocapsid protein with a molecular mass of approximately 29 kDa (this paper).

The ELISA and immuno-blot results indicated that PYSV is serologically distinct from PBNV, TSWV and INSV. The molecular mass of nucleocapsid protein of PYSV (29 kDa) is similar to that reported for serogroups I, II and III members and the estimation of 31 kDa by Reddy *et al.* (1991) is considered to be inaccurate. Serological relationships among the structural proteins have been used for classifying individual members within the family *Bunyaviridae*. It is apparent from the data obtained so far that members of tospoviruses could be distinguished on the basis of serological differences in N protein, indicating that the N protein is not conserved in the different serogroups. Evidence presented in this paper showed that the N protein of PYSV did not react with antibodies to TSWV (serogroup I), INSV (serogroup III) and PBNV (serogroup IV), in ELISA and immuno-blots analysis. The inability of the PYSV N protein to cross react with TSWV antiserum indicate that PYSV is not serologically related to serogroup II, since the N protein from members of serogroup II cross react with antisera against members of serogroup I.

The size of all the three PYSV RNAs is similar to that reported for other tospoviruses. Interestingly, a fourth RNA molecule of 1800 nucleotides was consistently detected in different preparations of nucleocapsids. This RNA was also observed in virus preparations purified from material collected at two different locations. It is possible that the 4th RNA species is defective RNA (D-RNA) of M RNA or L RNA, since D-RNAs have been observed in the M RNA of PBNV (Satyanarayana *et al.*, 1996*b*) and L RNA of TSWV (Resende *et al.*, 1992). But, the exact nature and origin of this molecule will only be apparent after it is sequenced and compared to the sequences of the L, M and S RNAs of PYSV.

In Northern blot hybridisations, none of the PYSV RNA species hybridised with cDNAs of the N gene, GP gene and the 3′ half of the RNA polymerase gene of PBNV indicating the absence of homology between PYSV and PBNV. In reciprocal tests, the cDNA clones of PYSV S and M RNAs did not hybridise with PBNV RNAs confirming the lack of homology between PYSV and PBNV. The GP gene probes of TSWV and INSV also failed to hybridise with any of the PYSV RNAs. The data obtained suggest that PYSV is distinct from PBNV, TSWV, and INSV. Based on these differences a lack of sequence homology would be expected between PYSV and other tospoviruses in serogroups I, III and IV.

PYSV has also been reported to occur in Thailand (Reddy *et al.*, 1991) and a virus resembling PYSV has been reported from Taiwan (Chen & Chiu, 1995). Data obtained *in*

preliminary experiments on PYSV at ICRISAT-IAC, Thailand and Taiwan suggest that PYSV is transmitted by the thrips *Scirtothrips dorsalis*. This information adds strength to our conclusion that for PYSV and viruses reported to be serologically related to it, a new serogroup should be established. Therefore we propose that the PYSV to be regarded as a distinct tospovirus, and placed in a different serogroup, tentatively designated serogroup V. Currently we are sequencing cDNA libraries representing the L, M and S RNAs of PYSV.

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References

Adam G, Yeh S D, Reddy D V R, Green S K. 1993. The serological comparison of tospovirus isolates from Taiwan and India with *Impatiens* necrotic spot virus and different tomato spotted wilt virus isolates. *Archives of Virology* **130**:237–250.

Bailey J M, Davidson N. 1976. Methylmercury as a reversible denaturing agent for agarose gel electrophoresis. *Analytical Biochemistry* **70**:75–85.

Chen C, Chiu R J. 1995. A tospovirus causing chlorotic fan-spot on peanut in Taiwan. In *International Symposium on tospoviruses and thrips of floral and vegetable crops, 7–11 November 1995, Taiwan Agricultural Research Institute, Taichung, Taiwan.* 18 pp. (Abstract).

de Avila A C, de Haan P, Kormelink R, Resende R de O, Goldbach R W, Peters D. 1993*a*. Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. *Journal of General Virology* **74**:153–159.

de Avila A C, de Haan P, Smeets M L L, Resende R de O, Kormelink R, Kitajima E W, Goldbach R W, Peters D. 1993*b*. Distinct levels of relationships between tospovirus isolates. *Archives of Virology* **128**:211–227.

de Haan P, Wagemakers L, Peters D, Goldbach R, 1990. The S RNA segment of tomato spotted wilt virus has an ambisense character. *Journal of General Virology* **71**:1001–1007.

de Haan P, de Avila A C, Kormelink R, Westerbroek A, Gielen J J L, Peters D, Goldbach R. 1992. The nucleotide sequence of the S RNA of *Impatiens* necrotic spot virus, a novel tospovirus. *FEBS Letters* **306**:27–32.

Elliot R M. 1990. Molecular biology of the *Bunyaviridae*. *Journal of General Virology* **71**:501–522.

Gibbs A J, Harrison B D. 1976. *Plant Virology: The principles*. London: Arnold. 292 pp.

Gubler U, Hoffman B J. 1983. A simple and very efficient method for generating cDNA libraries. *Gene* **25**:263–269.

Heinze C, Maiss E, Adam G, Casper R. 1995. The complete nucleotide sequence of the S RNA of a new tospovirus species, representing serogroup IV. *Phytopathology* **85**:683–690.

Hobbs H A, Reddy D V R, Rajeshwari R, Reddy A S. 1987. Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. *Plant Disease* **71**:747–749.

Kormelink R, de Haan P, Meurs C, Peters D, Goldbach R. 1992. The nucleotide sequence of the M RNA segment of tomato spotted wilt virus, a bunyavirus with two ambisense RNA segments. *Journal of General Virology* **73**:2795–2804.

Laemmli U K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature* **277**:680–685.

Law M D, Speck J, Moyer J W. 1991. Nucleotide sequence of the 3′ non-coding region and N gene of the S RNA of a serologically distinct tospovirus. *Journal of General Virology* **72**:2597–2601.

Law M D, Speck J, Moyer J W. 1992. The M RNA of *Impatiens* necrotic spot tospovirus (Bunyaviridae) has an ambisense genomic organization. *Virology* **188**:732–741.

Maiss E, Ivanova L, Breyel E, Adam G. 1991. Cloning and sequencing of the S RNA from a Bulgarian isolate of tomato spotted wilt virus. *Journal of General Virology* **72**:461–464.

Mohamed N A, Randles J W, Francki R I B. 1973. Protein composition of tomato spotted wilt virus. *Virology* **56**:12–21.

Murphy F A, Fauquet C M, Mayo M A, Jarvis A W, Ghabrial S A, Summers M D, Martelli G P, Bishop D H L. 1995. The classification and nomenclature of viruses: Sixth report of the International Committee on Taxonomy of *Viruses. Archives of Virology* **10** (Supplement).

Pang S Z, Slightom J L, Gonsalves D. 1993. The biological properties of a distinct tospovirus and sequence analysis of its S RNA. *Phytopathology* **83**:728–733.

Pang S Z, Bock J H, Gonsalves C, Slightom J L, Gonsalves D. 1994. Resistance of transgenic *Nicotiana benthamiana* plants to tomato spotted wilt and impatiens necrotic spot tospoviruses: Evidence of involvement of the N protein and N gene RNA in resistance. *Phytopathology* **84**:243–249.

Reddy D V R, Sudarshana M R, Ratna A S, Reddy A S, Amin P W, Kumar I K, Murthy A K. 1991. The occurrence of yellow spot virus, a member of tomato spotted wilt virus group, on peanut (*Arachis hypogaea* L.) in India. In *Virus-Thrips-Plant interactions of tomato spotted wilt virus*, pp. 77–88. Eds H T Hsu and R H Lawson. Proceedings of the USDA Agricultural Research Service, ARS-87.

Resende R de O, de Haan P, van de Vossen E, de Avila AC, Goldbach R, Peters D. 1992. Defective interfering L RNA segments of tomato spotted wilt virus retain both virus genome termini and have extensive internal deletions. *Journal of General Virology* **73**:2509–2516.

Sambrook J, Fritsch E F, Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor, USA: Cold Spring Harbor Laboratory Press.

Satyanarayana T, Mitchell S E, Reddy D V R, Brown S, Kresovich S, Jarret R, Naidu R A, Demski J W. 1996*a*. Peanut bud necrosis tospovirus S RNA: complete nucleotide sequence, genome organization and homology to other tospoviruses. *Archives of Virology* **141**:85–98.

Satyanarayana T, Mitchell S E, Reddy D V R, Kresovich S, Jarret R, Naidu R A, Gowda S, Demski J W. 1996*b*. The complete nucleotide sequence and genome organisation of the M RNA segment of peanut bud necrosis tospovirus and comparison with other tospoviruses. *Journal of General Virology* **77**:2347–2352.

Tas P W L, Boerjan M L, Peters D. 1977. The structural proteins of tomato spotted wilt virus. *Journal of General Virology* **36**:267–279.

Ullman D E, German T L, Sherwood J L, Westcot D M, Custer D M. 1993. Tospoviral replication in insect vector cells: Immunocytochemical evidence that the non-structural protein encoded by the S RNA of tomato spotted wilt tospovirus is present in the thrips vector cells. *Phytopathology* **83**:456–463.

Wijkamp I, Van Lent J, Kormelink R, Goldbach R, Peters D. 1993. Multiplication of tomato spotted wilt virus in its insect vector, *Frankliniella occidentalis*. *Journal of General Virology* **74**:341–349.

Yeh S D, Chang T F. 1995. Nucleotide sequence of the N gene of watermelon silver mottle virus, a proposed new member of the genus tospovirus. *Phytopathology* **85**:58–64.

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