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Bud necrosis of groundnut (*Arachis hypogaea*) in India caused by tomato spotted wilt virus*

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SUMMARY

Chlorotic ringspots or chlorotic leaf specking, terminal bud necrosis, axillary shoot proliferation and severe stunting of groundnut (*Arachis hypogaea*) were shown to be caused by tomato spotted wilt virus (TSWV).

All 28 species of plants tested were susceptible to the virus. Cowpea (*Vigna unguiculata* cv. C-152) was found to be a good assay host. TSWV remained infective in buffered sap of groundnut at a dilution of $10^{-2.5}$, after storage for 4 h at room temperature (30 °C) and for 10 min at 40 but not 45 °C.

The haemagglutination test was adapted to detect TSWV in crude extracts of groundnut. Sap from infected groundnut and tomato contained spherical membrane-bound virus particles 70 to 90 nm diameter. The virus was transmitted by thrips (*Scirtothrips dorsalis*).

The prevalence of TSWV in India and the high incidence in groundnut indicates that the virus is economically important.

INTRODUCTION

Although several virus diseases of groundnut (*Arachis hypogaea* L.) have been reported in India (Chohan, 1974; Raychauduri, 1977), none of the causal viruses except peanut mottle virus (PMV) has been characterised or compared with viruses infecting groundnuts in other countries (Reddy *et al.* 1978). Nariani & Dhingra (1963) described a mosaic disease of groundnut, and three virus diseases of groundnut ('bunchy top', 'chlorosis' and 'ring mottle') were reported by Sharma (1966). A bud necrosis or bud blight disease was reported by Reddy, Reddy & Appa Rao (1968) and Chohan (1972), and a ring mosaic disease similar to these two diseases was reported by Narayanasamy, Kandaswamy & Ramiah (1975). All these diseases were regarded as new by each of the authors, although most were similar in symptomatology and transmission characteristics. Chohan (1972) suggested that bud blight disease was caused by tomato spotted wilt virus (TSWV). We report here the symptomatology, transmission characteristics, host range, physical properties, serology and electron microscopy of the causal virus of bud necrosis or bud blight disease and its identification as TSWV.

MATERIALS AND METHODS

Several groundnut plants showing characteristic symptoms were collected during surveys and virus cultures were established in a screenhouse by graft-infecting healthy groundnut plants. Extracts from young leaves showing symptoms on such grafted plants were inoculated separately

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on cowpea, *Vigna unguiculata* (L.) Walp. cv. C-152) (Ghanekar & Nene, 1976), and the virus isolated from the local lesions was mechanically inoculated onto groundnuts. All healthy and infected plants were maintained in a screenhouse and were sprayed with 0·025% Metasystox (oxydemetonmethyl; Bayer, India) at weekly intervals.

Inoculation procedure. Inoculum was prepared in chilled buffer by grinding young infected leaves in a chilled mortar. Plants were dusted with Carborundum (600 mesh) and rubbed with the thick end of the pestle dipped in the extract. The buffer used was 0·05 M potassium phosphate buffer, pH 7·0 containing 0·02 M 2-mercaptoethanol (PBM). Other antioxidants such as 0·01 M sodium sulphite, 0·02 M sodium thioglycollate, 0·01 M dithiothreitol, 0·01 M cysteine hydrochloride, and the chelating agent 0·01 M sodium diethyldithiocarbamate were also effective but inferior to 0·02 M 2-mercaptoethanol as assessed by the number of local lesions produced on cowpea. Hence, for routine mechanical inoculation tests PBM was used for preparing inocula.

Assay host. Several plant species produced necrotic or chlorotic lesions, or both, on mechanically inoculated leaves. Since cowpea (cv. C-152) consistently produced more lesions within 4 to 5 days after inoculation, it was selected as an assay host. Fully expanded primary leaves were chosen for inoculation and all assays were performed using at least eight half-leaves for each treatment.

Host range. In host range studies, at least six plants of each species were inoculated and kept under observation for a minimum of 40 days in a screenhouse at temperatures ranging from 20 °C to 30 °C. All inoculated plants were checked for the presence of virus by back tests to cowpea.

Physical properties of the virus. The physical properties of the virus were determined in extracts from groundnut leaves prepared in PBM. For determining the thermal inactivation point, sap diluted to 10^{-1} was heated at various temperatures in a water bath for 10 min.

Thrips transmission. Plants raised in an 80 mesh (*c.* 0·33 mm/hole) brass cage were used in all transmission experiments. Healthy thrips colonies were raised from 10 adults collected from the field and maintained on groundnut plants by weekly transfer of nymphs. First or second instar nymphs were allowed acquisition feeds on detached infected groundnut leaves floated on water in a Petri dish. After an acquisition access period of 2–3 days, 10–15 nymphs were transferred to caged individual groundnut plants at the second or third quadrifoliate stage. After an inoculation feeding period of 12 days the thrips were killed by spraying with 0·025% Metasystox. The test plants were then kept in the screenhouse for observation, and those showing symptoms were later assayed on cowpea cv. C-152 and *Petunia hybrida* Vilm. cv. Coral Satin.

Seed transmission. Two thousand seeds from mechanically inoculated plants in the screenhouse and nearly 4000 seeds from naturally infected plants were sown and raised in sterilised soil in pots in an 80 mesh screenhouse. The plants were sprayed with 0·025% Metasystox at weekly intervals and were retained for 3 months. Seedlings suspected of having bud necrosis disease were tested for TSWV by inoculating cowpeas.

Haemagglutination test. Except where otherwise stated, details of the procedure for haemagglutination tests were similar to those described by Reddy *et al.* (1978). Ten volumes of tanned sheep blood cells at a 2·5% cell concentration, suspended in phosphate buffered saline (PBS), pH 6·4, were mixed with one volume of TSWV-antibody solution and incubated at 37 °C in a water bath for 30 min. The coated cells were resuspended in PBS, pH 7·2, containing normal rabbit serum diluted to 10^{-2} and bovine serum albumin at 0·2% concentration (nb PBS), washed twice in nb PBS and resuspended in the same solution to give a concentration of 2·5% of packed sensitised cells. Serial two fold dilutions of the test solutions were made in nb PBS and 0·5 ml was placed in each well (1·0 ml capacity) of a Lucite plate. Later 0·08 ml of sensitised cells (2·5%) was added to each well, mixed gently, incubated at room temperature for *c.* 1 h, and then kept at 4 °C for about 14 h. A positive haemagglutination reaction consisted of a definite

matting of cells with irregularity of the peripheral ring. In the case of a negative reaction the red cells formed a dense ring at the periphery of the well.

Electron microscopy. Pieces of healthy and infected groundnut and tomato leaves showing early and late symptoms were pre-fixed with 3% glutaraldehyde for 2 h at 4 °C, and post-fixed with 1% osmium tetroxide in 0·01 M potassium phosphate buffer, pH 7·3, for 2 h at 4 °C. The specimens were dehydrated in a graded series of acetone and then embedded in Epon 812. Sectioning was done with a Sorvall Porter Blum MT-2B ultramicrotome using glass knives. The sections were stained in 2% uranyl acetate and lead citrate (Reynolds, 1963) prior to examination with a Hitachi H-300 electron microscope.

RESULTS

Symptomatology in groundnut

Under field conditions, leaf symptoms first appeared about 40 days after planting, whereas those on mechanically inoculated plants, although similar, first appeared 10 to 15 days after inoculation. Symptoms initially consisted of distinct chlorotic ringspots or chlorotic speckling on the quadrifoliate leaf immediately below the terminal bud (Pl. 1, fig. 1). Sometimes concentric chlorotic ringspots, chlorotic ringspots with a few necrotic spots, or chlorotic leaf spots with green islands were seen on leaves, especially under field conditions. The fully expanded quadrifoliate leaf immediately below the terminal bud often became flaccid. Later, necrosis was observed on the petioles and along the stems, and sometimes on the terminal bud (Pl. 1, fig. 2). New leaves were smaller than normal and showed a wide range of symptoms including distortion, mosaic mottling and general chlorosis. Internodes were reduced in length and short axillary shoots with distorted and mottled leaves developed. Infected plants were stunted and bushy (Pl. 1, fig. 3), especially if infection occurred in the seedling stage. The kernels from infected plants were shrivelled and the testas were discoloured and mottled (Pl. 1, fig. 4).

Host range

The virus induced chlorotic and/or necrotic local lesions in *Beta vulgaris, Cajanus cajan* Accession No. ICRISAT 2376, *Chenopodium amaranticolor, C. quinoa* (Pl. 2, fig. 8), *Crotalaria juncea, Cucumis sativus* cv. National Pickling, *Gomphrena globosa, Nicotiana rustica, N. tabacum* 'Xanthi-nc', *Petunia hybrida* cv. Coral Satin (Pl. 2, fig. 6), and chlorotic or necrotic spots followed by systemic infection in *Canavalia ensiformis, Datura stramonium, Dolichos uniflorus, Glycine max* cv. Bragg, *Lycopersicon esculentum* cvs Pusa Ruby and Perfection (Pl. 2, fig. 7), *Nicotiana clevelandii, N. glutinosa*, a hybrid between *N. glutinosa* X *N. clevelandii, Phaseolus vulgaris* cvs Bountiful and Topcrop, *P. lunatus* cv. Henderson Bush Lima, *Physalis floridana, Pisum sativum* cv. Bonneville, *Vigna radiata* cv. Hy-45, *V. mungo* cv. UPU-1, *V. unguiculata* cvs C-152 and California Black Eye (Pl. 2, fig. 5), *Vinca rosea, Zinnia elegans.*

Thrips transmission

Adults of *Scirtothrips dorsalis* Hood collected from plants with bud necrosis successfully transmitted TSWV to 14 of 65 plants. Similarly, thrips that acquired virus from infected leaves and were then allowed inoculation feeds of 12 to 15 days transmitted the disease to 31 out of 164 groundnut plants. None of the 160 plants exposed to thrips from healthy colonies were infected.

Seed transmission

Of nearly 6000 seeds collected from the infected plants, 1800 seeds (30%) produced normal plants, 540 seeds (9%) produced malformed plants and the remainder failed to germinate. None of the plants developed disease symptoms, and assays on cowpea from the malformed stunted plants gave negative results. The results indicate that the virus was not seedborne.

Physical properties of the virus in buffered sap

The infectivity dilution end-point was between $10^{-2.5}$ to $10^{-3.0}$ and the thermal inactivation point between 45 °C to 50 °C. Leaf extracts retained infectivity for 4 h but not for 5 h at room temperature (30 °C).

Haemagglutination tests

In two experiments, antiserum received from Dr Van Regenmortel had a haemagglutination titre of 1/3200 with extracts from infected leaves and of 1/200 to 1/400 with healthy leaf extracts. In three experiments, TSWV antiserum received from Dr Gooding had a maximum titre of 1/500 with healthy leaf extracts, and titres of 1/20 480 to 1/32 000 with infective leaf extracts (Table 1).

Table 1. *Haemagglutination titres of healthy and bud-necrosis infected groundnut leaf extracts*

		Haemagglutination titres with different antisera†				
		TSWV antisera				
Expt no.	Extracts*	Van Regenmortel	Gooding	PMV	PSV	Normal rabbit serum
1	Healthy	200	NT‡	—§	—	—
	Infected	3200	NT	—	—	—
2	Healthy	400	—	—	—	—
	Infected	3200	—	—	—	—
3	Healthy	NT	320	—	NT	—
	Infected	NT	20 480	—	NT	—
4	Healthy	NT	400	—	NT	NT
	Infected	NT	25 600	—	NT	NT
5	Healthy	NT	500	NT	NT	—
	Infected	NT	32 000	NT	NT	—

* Extracts were prepared in phosphate buffered saline, pH 7·2, containing normal rabbit serum and bovin-serum albumin. Serial two-fold dilutions were tested starting from 1/50 dilution.

† Titres represent the reciprocal of the highest reacting dilution.

‡ NT, not tested.

§ Positive reactions were not detected in all the dilutions tested.

Cells sensitised with normal rabbit serum, peanut mottle virus antiserum or peanut stunt virus antiserum (from the American Type Culture Collection, PVAS 39) did not react with any of the dilutions of extracts from healthy or infected leaves.

Electron microscopy

Spherical membrane-bound virus particles were observed in the cytoplasm of infected leaves from groundnut and tomato. The particles were 70 to 90 nm in diameter. Within infected cells, some of the particles were present in the cisternae of the endoplasmic reticulum (Pl. 3, fig. 9) and in some cases were present in membranous bags.

DISCUSSION

Our results show that the bud necrosis disease of groundnut in India is caused by TSWV on the basis of various criteria (Best, 1968) for a positive identification of TSWV.

The results of our haemagglutination tests indicated that both the TSWV antisera tested

contained a small proportion of antibodies to healthy plant antigens. Nevertheless, the titres obtained with infected leaf extracts were at least 8-fold and 100-fold higher than those from healthy leaves using antisera from Van Regenmortel and Gooding, respectively. Although our results agree with those of Paliwal (1974) and Tas, Boerjan & Peters (1977) that TSWV antisera react with healthy sap, high virus titres were also obtained in this investigation. The haemagglutination test is known to detect smaller quantities of antigen (Saito & Iwata, 1964; Abu Salih, Murant & Daft, 1968; Reddy, Hariprasadarao, Padma & Gopal Rao, 1969) than other serological tests, and it is thus preferable for diagnosing TSWV.

Particle morphology observed here in thin sections resembled that reported for TSWV (Black, Brakke & Vatter 1963; Gumpf & Weathers, 1972; Halliwell & Philley, 1974; Milne, 1970; Paliwal,1974).

Poor germination of seeds from TSWV infected plants has been reported previously (Helms, Grylls & Purss, 1961; Halliwell & Philley, 1974) and confirmed in this investigation. The seeds from infected plants are generally small and shrivelled and this is probably the reason for their poor germination.

The discovery of a new vector, *Scirtothrips dorsalis*, is important. Thus, this newly recognised vector of TSWV has a very wide host range (Ananthakrishnan, 1973) and many of these hosts are susceptible to TSWV. It will be interesting to test *Thrips tabaci* (Lind.) and *Frankliniella* spp. as possible vectors of TSWV in India. In addition, other thrips infesting groundnut (*Caliothrips indicus* Bagnall, *Megalurothrips distalis* (Karny)) need to be tested for their ability to transmit TSWV.

The diseases described from India by Nariani & Dhingra (1963), Chohan (1972) and Narayanasamy *et al.* (1975) under different names are all possibly caused by TSWV. Symptom expression is influenced by such factors as cultivar, age of plant, physiology of plant, amount of inoculum introduced and prevailing climatic conditions, particularly temperature. Plants infected early usually show bud necrosis, whereas those infected later might not. The mosaic disease reported by Nariani & Dhingra (1963) is similar to that reported by us. Moreover, their virus was not transmissible by aphids (*Aphis craccivora* Koch, *Aphis gossypii* Glov.), whiteflies (*Bemisia tabaci* Genn.), or leaf hoppers (*Empoasca devastans* Dist., *Orosius* sp.), but they did not test thrips. In our experience, mechanical transmission was not always successful even when sodium sulphite was incorporated in the extractant. For successful transmission of TSWV the following conditions must be met (1) young infected groundnut leaf tissue showing chlorotic spots should be used as a source of inoculum, (2) test plants should be of a suitable age; for instance, fully expanded primary leaves of cowpea and groundnut plants up to third or fourth quadrifoliate stage, (3) inoculum should be prepared in a chilled extractant with a cold pestle and mortar.

Sharma (1966) reported three diseases of groundnut from India. Bunchy top and ring mottle diseases resemble those caused by TSWV and were not transmitted by the same group of insects employed by P. W. Amin *et al.* (in preparation). Sharma (1966) reported that both the diseases were transmitted through seed. However, the number of seeds used in his transmission studies were low and, in addition, external infestation by viruliferous thrips was possible. The reported cross-protection experiments were not sufficiently conclusive to consider bunchy top and ring mottle as two independent viruses. It is possible therefore that bunchy top and ring mottle diseases are caused by TSWV.

Narayanasamy *et al.* (1975) described ring mosaic as a new disease of groundnut, but the symptoms described were similar to those observed previously by Reddy *et al.* (1968), Chohan (1972) and by us. Further, the virus from the infected plants from Coimbatore produced symptoms on *Petunia*, cowpea and groundnut similar to those by TSWV. Therefore, the disease reported by Narayanasamy *et al.* (1975) was probably caused by TSWV.

TSWV in groundnut was earlier reported from Brazil (Costa, 1941; 1950), South Africa

(Dyer, 1949; Klesser 1966), Australia (Helms *et al.* 1961) and USA (Halliwell & Philley, 1974). Our findings confirm its occurrence on groundnut in India.

TSWV has not been found to cause severe losses to groundnut crops in Brazil, South Africa and USA. However, yield losses of up to 90% have been reported from Australia (Saint-Smith *et al.*, 1972). The reports published in India (Chohan, 1974) indicate that TSWV is probably the most important virus disease of groundnut, and crop losses of up to 80% have been reported. Our recent surveys indicate infection rates ranging from 5% to 80%, and the disease is present in every groundnut growing area of India. Purification of the virus, epidemiology of the disease and large scale screening of exotic and Indian germplasm for sources of resistance are in progress.

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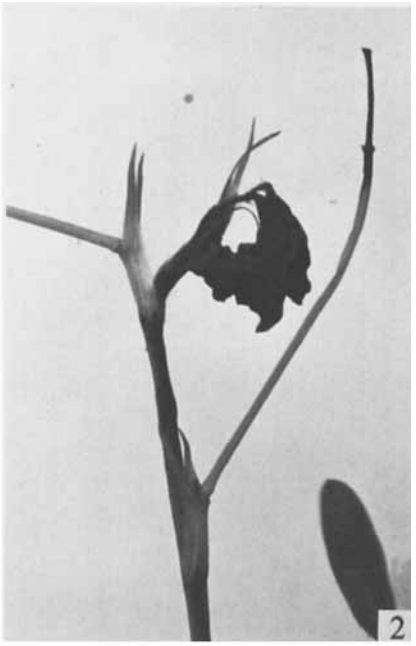
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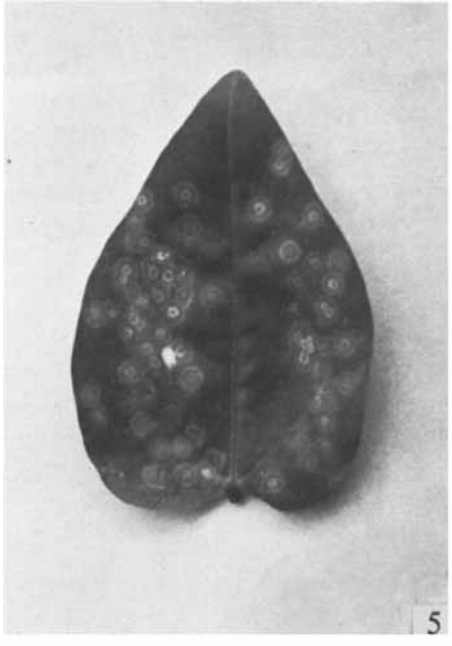
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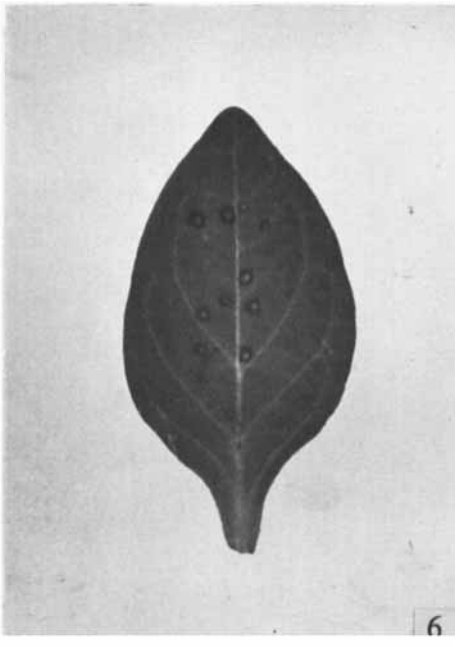
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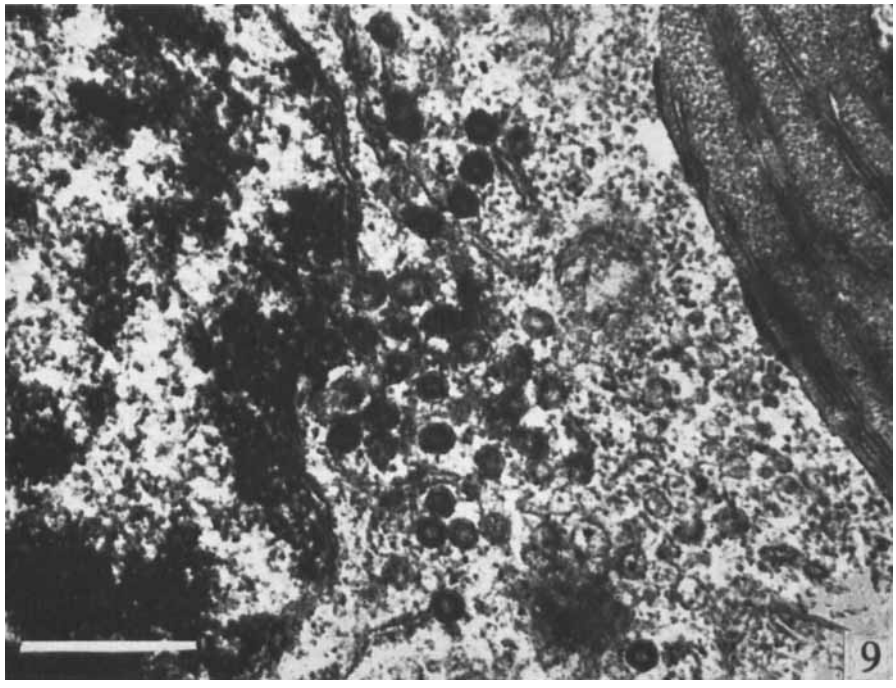
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EXPLANATIONS OF PLATES

PLATE 1

Fig. 1. Chlorotic and necrotic rings induced by TSWV in inoculated leaves of *A. hypogaea* cv. TMV 2.

Fig. 2. *A. hypogaea* cv. TMV 2 showing terminal bud and stem necrosis 12 days after inoculation with TSWV.

Fig. 3. Axillary shoot proliferation, severe leaf deformity and stunting induced by TSWV on *A. hypogaea* cv. TMV 2.

Fig. 4. Shrivelled and mottled kernels of *A. hypogaea* cv. TMV 2 from early infected plant (Top); kernels from uninfected plants (Bottom).

PLATE 2

Fig. 5. Concentric chlorotic lesions in primary leaves of *V. unguiculata* cv. C-152 induced by TSWV.

Fig. 6. Necrotic local lesions on *P. hybrida* cv. Coral Satin induced by TSWV.

Fig. 7. Concentric necrotic rings in inoculated leaves of *L. esculentum* cv. Pusa Ruby induced by TSWV.

Fig. 8. Necrotic local lesions in *C. quinoa* leaves induced by TSWV.

PLATE 3

Fig. 9. Electron micrograph of thin section of a leaf of TSWV-infected *A. hypogaea* cv. TMV 2. Bar represents 500 nm.