Groundnut Virus Research at ICRISAT

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Several virus diseases of groundnut have been reported in India based on symptoms, host range and biological properties. These properties are now regarded as inadequate to identify a virus. Characterization should be based on serology, electron microscopy, transmission and physico-chemical properties.

Three economically important virus diseases (bud necrosis, clump and peanut mottle) and several virus diseases of minor importance in India have now been fully characterized.

Bud Necrosis Disease

Bud necrosis disease caused by tomato spotted wilt virus (TSWV) has been recognized as one of the most important virus diseases of groundnuts in India (Chohan 1974; Ghanekar et al. 1979). The disease has also been reported on groundnuts in several other countries including Brazil, USA, S. Africa and Australia (Costa 1941; Halliwell and Philley 1974; Klesser 1966; Helms et al. 1961). A clear account of the disease symptoms was given by Reddy et al. (1968).

The causal virus was characterized at ICRISAT (Ghanekar et al. 1979) and the thrips vector chiefly responsible for transmitting the disease was identified (Amin et al. 1978). Bud necrosis has been shown to cause yield losses of up to 50% and occurs in all the major groundnut growing areas of India. The incidence ranges from 5 to 80% in different parts of the country (Chohan 1972; Ghanekar et al. 1979).

Symptoms on Groundnut

The typical disease symptoms on groundnut include chlorotic rings, terminal bud necrosis, severe stunting, proliferation of axillary shoots with deformed leaves and production of discolored and shrivelled kernels.

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Diagnostic Hosts

The virus produces chlorotic and necrotic local lesions on *Vigna unguiculata* (cowpea cv C-152) and necrotic local lesions *onPetuniahybrida* (cv Coral Satin) which do not become systemic.

Host Range

The virus was found to have extremely wide natural and experimental host ranges. *Vigna radiata* (cv Hy-45), *Vigna mungo* (cv UPU-1), *Phaseolus vulgaris* (cv Local), *Vicia faba, Lycopersicon esculentum* (cv Pusa Ruby) and *Pisum sativum* were all susceptible to infection by TSWV. In addition a number of weeds commonly encountered in groundnut fields were also susceptible.

Biological Properties

The virus has a thermal inactivation point at 46℃ and the longevity in vitro is approximately 5 hours at 25℃. These properties indicated that bud necrosis could be related to tomato spotted wilt virus.

Electron Microscopy

Thin sections of groundnut leaves under the electron microscope showed membrane bound virus particles 70-90 nm in diameter and were associated with the endoplasmic reticulum. These particles resemble those of TSWV.

Serology

Antisera for TSWV obtained from the USA and S. Africa when used in haemagglutination tests clearly revealed the presence of viral antigens in crude bud necrosis infected groundnut extracts.

Transmission

The virus was mechanically sap transmissible from plant extracts prepared in 0.05M potassium phosphate buffer (pH 7.0) containing 0.02M 2-mercaptoethanol added as an antioxidant. It was consistently transmitted by *Frankliniella schultzei* (Trybom) and to a lesser extent by *Scirtothrips dorsalis* Hood. The virus was not transmissible through seed of groundnut (Ghanekar et al. 1979).

Control

Screening for Disease Resistance

So far nearly 7000 germplasm lines of *Arachis hypogaea* have been screened under high natural disease incidence in the field and none showed any marked resistance to the virus. However, the cultivars Robut 33-1 and NC Acc 2575 consistently showed lower than average incidence of the disease under field conditions.

Several wild *Arachis* species have been screened under high natural disease incidence in the field, and also by mechanical sap inoculation in the screenhouse. So far *Arachis chacoense, A. glabrata, Arachis* sp (PI 262848) and *Arachis pusilla* have not become infected in thesetests, butthese results need confirmation.

Cultural Practices

As sources of resistance are still being sought, efforts *are* being concentrated on the development of cultural practices to control the disease.

Experiments on effects of date of sowing, plant spacing and intercropping with pearl millet on disease incidence are giving promising results. Early planting at the onset of the rainy season decreased disease incidence and reduced losses from bud necrosis disease. Planting at high density also reduced disease incidence (Table 1).

Experiments on the effect of intercropping with pearl millet were started recently and preliminary observations show a lower disease incidence in the intercropped situation when compared with the sole crop.

Peanut (Groundnut) Clump Virus

A disease of groundnuts resulting in severely stunted plants with small, dark green leaves was observed in 1977 in crops grown in the sandy soils of Punjab and Gujarat. Most of the infected plantsfailed to produce pods, and even in cases of late infection, losses of up to 60% were recorded. A sap transmissible virus which reproduced the disease symptoms was isolated and is being characterized.

Symptoms on Groundnut

Infected plants are severely stunted with small dark green leaves. The young quadrifoliate leaves show mosaic mottling and chlorotic rings. Roots become dark colored and the outer layers peel off easily.

Table 1. Effect of plant spacing on the incidence of bud necrosis disease (TSWV).

Interrow and Intrarow plant spacing (cm)	Postrainy season 1978--79		Postrainy season 1979--80	
	Disease (%)	Yield (kg/ha)	Disease (%)	Yield (kg/ha)
37.5 x 5.0	10	3493	7	2153
37.5 x 15.0	23	2855	16	1524
75.0 x 5.0	20	2289	9	1570
75.0 x 15.0	40	1745	18	917
150.0 x 5.0	23	1270	11	740
150.0 x 15.0	43	777	21	409

Diagnostic Hosts

Phaseolus vulgaris (cv Local), on which the virus produces veinal necrosis, and *Canavalia ensiformis,* on which discrete necrotic lesions with chlorotic centers are produced, have been identified as diagnostic hosts.

Host Range

The virus has an extremely wide host range and several weeds commonly occurring in groundnut fields are also infected by the virus.

Biological Properties

The thermal inactivation point of the virus is between 60° and 65°C and longevity in vitro is 2-3 days at room temperature.

Purification

Nicotiana hybrid *(N. clevelandii* x *N. glutinosa)* consistently gives high virus concentrations. A method to purify the virus from *crude Nicotiana* hybrid leaf extracts has been successfully devised. Polyethylene glycol precipitates of chloroform treated infected leaf extracts are subjected to density gradient centrifugation in sucrose solutions. Virus obtained from the gradients can be inoculated onto healthy groundnut plants and diagnostic hosts where it produces typical symptoms.

Electron Microscopy

Purified virus preparations, and leaf dips of infected leaves of groundnut and *Nicotiana* hybrid, revealed the presence of rod-shaped virus particles of 200-500 nm in length, and 23-25 nm in width, with a central hollow core.

Serology

Antisera were obtained of strains of the soilborne tobacco rattle and pea early browing viruses which have particle morphology similar to the clump virus. These were tested against crude plant extracts and purified extracts of clump virus but there was no positive reaction.

Transmission

The virus was successfully transmitted by means of mechanical inoculations and grafting.

The following observations suggested that the virus was soilborne and possibly transmitted by nematodes: (1) the disease was restricted to sandy soils; (2) infected plants could be obtained by sowing healthy seeds in soil samples collected from depths of 12-28 cm in infected fields; (3) the disease occurred in patches in the field and reappeared in the same positions in succeeding years; (4) air-dried soil could not reproduce the disease; and (5) nematocide applications to infested soils reduced the incidence and spread of the disease.

Nematodes isolated from infested soils, and inoculated onto healthy plants grown in sterilized soil produced the disease in some recent tests. These results need to be confirmed.

Relationship with Similar Viruses Reported on Groundnuts

Based only on symptoms, Sundararaman (1927) described a similar disease in India, which he named clump.

The symptoms observed also resemble those of clump disease reported from West Africa (Germani et al. 1975). In both cases the disease was soilborne and application of Nemagon reduced the disease (Germani et al. 1973). Both diseases are caused by viruses with similar particle structure (Germani et al. 1975; Thouvenel et al. 1976). However, both viruses have to be tested serologically before the relationship between them can be confirmed.

Control

Nematocide and Fungicide Treatments

In collaboration with the Oilseeds Section of Punjab Agricultural University, the nematocides Nemagon, Carbofuran, Temik and a mixture of the fungicides Bavistin and Blitox, were tested for their effect in controlling the disease. Untreated plots served as controls. The chemicals were applied to the soil 1 week before planting and the susceptible cultivar M-13 was used. Nemagon and Temik were the most effective in reducing the disease incidence and increasing the yield when compared with untreated plots.

Screening for Disease Resistance

Screening was carried out in infected soils of the Punjab where the disease had been recurring for three consecutive years. The plots selected had shown up to 98% incidence of the disease in the previous season. A susceptible cultivar M-13 was sown after every 10 test cultivars. Eight cultivars (M 884-75, C 334-AB-13, NC Acc 17847, NC Acc 17866, NC Acc 17732, NC Acc 17740, NC Acc 17840, and EC 21887) showed no disease symptoms.

Another ten cultivars showed a very low incidence of visibly diseased plants. These cultivars will be retested under field and laboratory conditions before any conclusions on their possible resistance or tolerance can be drawn.

Peanut (Groundnut) Mottle Virus

Peanut mottle virus (PMV) is widespread and has been positively identified in the USA (Kuhn 1965), E. Africa (Bock 1973), Australia (Behncken 1970), Europe (Schmidt et al. 1966), Japan (Inouye 1969), the Philippines (Benigno et al. 1977), South America (Herold et al. 1969), West Malaysia (Geh et al. 1973) and India (Reddy et al. 1978). The disease also appears to be present in China (Gibbons, personal communication). The disease can cause up to 30% loss in yield (Kuhn et al. 1975).

Symptoms on Groundnut

Newly formed leaves show mild mottling and vein clearing, whereas older leaves show upward curling and interveinal depression with occasional dark green islands. Infected plants are not severely stunted and older plants seldom show typical disease symptoms.

Diagnostic Host

The virus produces reddish brown necrotic lesions on inoculated leaves of *Phaseolus vulgaris* (cv Topcrop) which was found to be a good diagnostic host for the virus.

Host Range

The virus has a narrow host range and infects mostly legumes.

Biological Properties

The virus has a thermal inactivation point between 55° and 60°C and longevity in vitro is 48 hours at 25°C.

Purification and Antiserum Production

The virus has been successfully purified employing a method developed at ICRISAT (lizuka et al. in preparation). An antiserum has been produced by injecting purified virus preparations into rabbits.

Electron Microscopy

Purified virus preparations and sections of infected leaves, when observed under the electron microscope, reveal the presence of long, flexuous, rod-shaped particles of 700 nm in length.

Serology

An antiserum obtained from the USA, and one produced at ICRISAT, were reacted with PMV using agar gel diffusion, haemagglutination and Enzyme Linked Immuno Sorbant Assay (ELISA) tests. Positive results were obtained in all tests for PMV.

Transmission

The virus is seed transmitted in a range from 0.1 to 3.5% depending on the groundnut cultivars.

Aphis craccivora and *Myzus persicae* transmit the virus in a stylet-borne (non-persistent) manner.

Control

Screening for Disease Resistance

The natural incidence of PMV is not high enough for meaningful screening of cultivars for resistance in the field. It was therefore necessary to reproduce the disease on a large scale underfield conditions. A spray inoculation technique has been developed in which inoculum is mixed with celite and sprayed through fine nozzles at 50 PSI. About 1000

plants can be inoculated in one hour and about 80% of the plants become infected.

An earlier report indicated that no immunity had been found to peanut mottle virus (Kuhn 1968) in groundnut cultivars from different parts of the world. However, tolerance of some cultivars to PMV where there is no reduction in yield even though plants became infected, was reported (Kuhn et al. 1978). Using the inoculation technique described, about 200 cultivars have been screened sofarand yield losses have been estimated. None of the cultivars tested showed immunity or tolerance to PMV.

Screening Cultivars which do not Transmit the Virus Through the Seed

Diseased plants with infected seeds are the primary sources of inoculum. The secondary spread is by aphids which acquire the virus from plants infected through seeds. It would be desirable to have a cultivar which did not transmit the virus through the seed. Approximately 1000 seeds were obtained from infected plants of a range of cultivars. So far two cultivars, EC 76446 (292) and PI 259747, have not shown any seed transmission. Over 5000 seeds from infected plants of these cultivars will soon be tested under field conditions.

Virus Diseases of Minor Importance

Cowpea mild mottle virus (CMMV) and peanut green mosaic virus (PGMV) have been characterized on the basis of electron microscopy, serology, chemical characteristics and host range. CMMV has been detected occurring naturally in the Punjab, Andhra Pradesh and Uttar Pradesh but the incidence is less than 1%. PGMV has so far been detected only in the Chittoor district of Andhra Pradesh.

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