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Identification of Peanut Green Mosaic Virus Strains in India*

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With 3 figures

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Abstract

During field surveys, three peanut green mosaic virus isolates differing in symptomatology on groundnut and a few other hosts were collected. Ultrathin sections of infected groundnut leaflets showed cytoplasmic inclusions with pin wheels and scrolls. In enzyme-linked immunosorbent assay they reacted strongly with antisera to peanut green mosaic and soybean mosaic virus antisera, and moderately with adzuki bean mosaic and peanut stripe virus antisera. All isolates also reacted positively with antisera to peanut eye spot, blackeye cowpea mosaic, pea seed-borne mosaic, potato virus Y and tobacco etch viruses, and did not react with antisera to peanut mottle, bean yellow mosaic, bean common mosaic, clover yellow vein and sugarcane mosaic viruses. SDS-PAGE analysis of purified virus preparations of the three isolates showed a single polypeptide with mol. wt. of 34,500 daltons. Based on these results, the three isolates are identified as biologically distinct strains of peanut green mosaic virus.

Zusammenfassung

Die Identifizierung von peanut green mosaic virus-Isolaten in Indien

Gesammelt wurden drei peanut mosaic virus-Isolate während Feldbesichtigungen, die sich in ihrer Symptomatologie an Erdnüssen und einigen anderen Wirtspflanzen unterschieden. Ultradünne Schnitte infizierter Erdnußblättchen zeigten das Vorhandensein von cytoplasmatischen Einschlüssen mit Drehscheiben und Schnörkeln. In ELISA reagierten sie sehr stark mit peanut green mosaic- und

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soybean mosaic-Virusantisera, und mittelstark mit Antisera gegen adzuki bean mosaic- und peanut stripe virus. Alle drei Isolate reagierten auch mit Antisera gegen peanut eye spot, blackeye cowpea mosaic, pea seed-borne mosaic, potato virus Y and tobacco etch virus positiv, jedoch nicht mit Antisera gegen peanut mottle, bean yellow mosaic, bean common mosaic, clover yellow vein und sugarcane mosaic virus. Eine SDS-PAGE-Analyse der gereinigten Viruspräparate der drei Isolate zeigte das Vorhandensein eines einzelnen Polypeptids mit einem Molekulargewicht von 34 500 daltons. Basierend auf diesen Ergebnissen werden die drei Isolate als biologisch verschiedene Stämme des peanut green mosaic virus identifiziert.

Several viruses infecting groundnut in India have been characterized (IIZUKA and REDDY 1986, REDDY 1988). Of these, three belong to potyvirus group. They are peanut mottle (PMV), peanut stripe (PStV) and peanut green mosaic viruses (PGMV). PMV and PStV were observed in different parts of the country (REDDY 1988, PRASADA RAO *et al.* 1988), whereas PGMV was observed only in Chittoor district of the state of Andhra Pradesh. During our field surveys of groundnut crop in Andhra Pradesh, we have collected three virus isolates which were symptomatologically distinct from viruses currently known to occur on groundnut in India. They could be distinguished on the basis of symptoms produced on *Phaseolus vulgaris* L. cv. Local. Although all the three isolates produced local lesions on inoculated primary leaves, one isolate was non-systemic (NS), another produced systemic mosaic (SM) and the third one produced systemic veinal necrosis (SN). Aphid transmission of these isolates was reported earlier (SAILAJA *et al.* 1986). We report in this paper host range and symptoms, serological relationships and molecular mass of capsid protein of NS, SM and SN isolates of PGMV.

Materials and Methods

The three isolates, showed symptoms on groundnut (*Arachis hypogaea* L.) which were different from the PGMV reported by SREENIVASULU *et al.* 1981. They were first established in a screen house by graft-inoculating healthy groundnut cv. TMV2. They were then mechanically transmitted to groundnut and French bean (*Phaseolus vulgaris* L. cv. Local) with extracts prepared in 0.05 M phosphate buffer, pH 7.0, containing 0.02 M 2-mercaptoethanol (PBM). After five successive passages of virus from a single lesion produced on *P. vulgaris*, each isolate was maintained on groundnut.

At least six plants of each test species or cultivar were inoculated at the same time in host range studies. Irrespective of the symptoms produced, inoculated and subsequently produced leaves were tested by enzyme-linked immunosorbent assay (ELISA) utilizing homologous antisera and by local lesion assay utilizing *Chenopodium amaranticolor* Coste and Reyn.

Physical properties were studied in crude sap of groundnut extracted and diluted to 10^{-1} in PBM. To determine the thermal inactivation point, 1 ml of sap was heated in a water bath for 10 min at various temperatures. Longevity *in vitro* at room temperature (25 °C) was tested at 24 h intervals.

The three isolates were purified from white lupins (*Lupinus albus* L.) by the method described for peanut stripe virus (DEMSKI *et al.* 1984). The virus pellets were suspended in 0.01 M phosphate buffer, pH 7.2, and stored at −70 °C for further analysis.

For capsid protein subunit molecular mass determination, purified preparations of the three isolates were solubilized by suspending in 0.0625 M Tris-HCl buffer, pH 6.8, containing 2 % sodium dodecyl sulphate (SDS) and 1 % 2-mercaptoethanol and heating at 100 °C for 2 min. SDS-polyacrylamide gel electrophoresis (PAGE) was performed in a 10 % resolving gel with a 3.3 % spacer gel using the Laemmli discontinuous buffer system (LAEMMLI 1970). Gels were stained with Coomassie Blue R-250 and the relative mol. wt. of the capsid protein subunit estimated using SDS-PAGE low mol. wt. standards (Bio-Rad).

Serological relationships of the three isolates with PGMV and other potyviruses were determined in double immunodiffusion and direct antigen coating form of ELISA (DAC-ELISA). Various heterologous antisera used were generous gifts from others working with potyviruses (soybean mosaic virus [SMV] and back-eye cowpea mosaic virus [BICMV] antisera were from Dr. D. E. PURCIFULL, U.S.A.; Azuki bean mosaic virus [AZBMV], bean common mosaic [BCMV] potato virus Y [PVY], and tobacco etch virus [TEV] antisera were from Dr. N. IIZUKA, Japan; pea seed-borne mosaic virus [PSBMV] antiserum was from Dr. L. BOS, Netherlands; peanut stripe virus [PStV] antiserum was from Dr. J. W. DEMSKI and groundnut eye spot virus [GEV] antiserum was from Dr. J. M. DOLLET, France). SDS-immunodiffusion experiments were performed in agarose gels prepared according to PURCIFULL and BATCHELOR (1977). Well arrangement consisted of six peripheral wells around a central well. The central well was filled with antiserum about 15 min prior to the filling of the peripheral wells with antigens. The latter consisted of purified virus suspensions (0.5 mg ml^{-1}). Readings were recorded 24 h later.

DAC-ELISA was performed as described by HOBBS *et al.* (1987). The antigen samples, extracted in 0.05 M carbonate buffer, pH 9.6, containing 0.01 M DIECA, were squeezed through 2 layers of muslin cloth, and used at 10^{-2} dilution. In the subsequent steps, the crude antisera and rabbit Fc-specific globulins conjugated to alkaline phosphatase were used at 10^{-4} dilution in conjugate buffer (PBS-Tween-PVP-Ovalbumin). Finally, p-nitrophenyl phosphate (Sigma) was added at 0.25 mg/ml and incubated for 30 min at room temperature. The absorbance readings (A410nm) were taken with a Dynatech Micro-ELISA plate reader. Values were deducted from buffer controls. Three independent experiments were conducted with a fresh set of antigens for each experiment.

Virus particle morphology and modal length for each isolate were determined by trapping the virus particles in crude extracts with PGMV antiserum at 1 : 5000 dilution. Particles were stained with 1 % uranyl formate and observed under a Philips Model 201C electron microscope. Inclusion body morphology was determined for each isolate. Peanut tissue systemically infected with each isolate was fixed and processed for thin sectioning and electron microscopy as described by REDDY *et al.* (1983).

Results and Discussion

The three isolates did not produce any symptoms on the inoculated groundnut leaflets. Newly developed leaflets, 10—12 days after inoculation, showed chlorotic spots and veinal chlorosis with all the three isolates. However, these symptoms became inconspicuous as these leaflets matured. Nevertheless, subsequently developed leaflets showed distinct symptoms according to each isolate: NS isolate produced mosaic consisting of large dark and light green areas (Fig. 1) with no effect on leaf size and plant growth; SM isolate produced mosaic consisting of chlorotic areas and spots (Fig. 2), with reduced leaflet size and plant growth; SN isolate produced vein clearing, oak-leaf mosaic (Fig. 3) with severe reduction in leaflet size and plant height. Limited trials on yield loss estimates showed that SM and SN isolates caused severe reduction in plant growth and in yield of pods (SATYANARAYANA 1987).

The three isolates incited distinct symptoms in a few host species and similar symptoms in others (Table 1). When compared to the host range data of other groundnut virus diseases reported from India (IIZUKA and REDDY 1986, PRASADA RAO *et al.* 1988, REDDY 1988), these isolates closely resemble PGMV. The main differences with PGMV were in symptoms produced in groundnut, French bean and soybean (*Glycine max* L.) but not in other host plants tested. The three isolates had a similar thermal inactivation point (between 55°—60 °C) and infectivity dilution end point (between 10^{-3}—10^{-4}). The NS isolate retained infectivity for 2 days, SN isolate for 3 days and SM isolate for 4 days at 25 °C. The virus

Fig. 1. (top, left) NS isolate infected groundnut leaf with mosaic and chlorotic rings

Fig. 2. (top) SM isolate infected groundnut leaf showing whitish spots and general chlorosis

Fig. 3. (left) SN isolate infected groundnut leaf with oak leaf mosaic symptoms

particles of the three isolates were flexuous rods with a modal length of c. 750 nm (average of 100 particles for each isolate). In thin sections of infected groundnut leaflets, all the three isolates produced cytoplasmic inclusion bodies in the form of pinwheels and scrolls. In SDS-PAGE, purified preparations of the three isolates revealed a single polypeptide with an estimated mol. wt. of 34,500 daltons. Polypeptide from purified PGMV preparation co-migrated with capsid proteins of all the three isolates.

In double immunodiffussion tests, all the three isolates gave a strong confluent reaction with PGMV antiserum and no visible reaction with PStV and PMV antisera. Other antisera were not tested in gel diffusion tests. But in ELISA all the three have reacted with PStV antiserum (Table 2). In DAC-ELISA, the reaction of the three isolates with several potyvirus antisera was studied to ascertain their relationship with other potyviruses. As shown in Table 2, all isolates reacted strongly with PGMV, and SMV antisera, moderately with AZBMV and PStV antisera. All isolates also cross reacted with BlCMV, GEV, PVY, PSBMV and TEV antisera. SM isolate consistently gave higher absorption values as compared with NS and SN isolates which we attribute to higher SM

concentration in groundnut tissue. Nevertheless the reaction pattern was comparable among them. All isolates failed to react with antisera to PMV, BCMV and clover yellow vein (CYV) virus antisera. Although PGMV was considered to be a distinct potyvirus infecting groundnut (SREENIVASULU *et al.* 1981), its serological relationships with only PMV were studied. Data presented show that PGMV is distinct from PMV as was reported earlier using agar gel double diffusion and haemagglutination tests. Though it reacted with PStV and GEV, unlike PStV, PGMV failed to react with CYVV antiserum.

The results show that each of the three PGMV strains could be differentiated on the basis of their reaction on selected host plants. They differ from PGMV by

Table 1

Comparative host-ranges of the three isolates of peanut green mosaic virus

Host[a]	Symptoms[b]			
	Type PGMV	NS	SM	SN
Phaseolus vulgaris				
'Local'	NLL	NLL	NLL, SM	NLL, SN
'Top crop'	—	—	—	—
'Dark Red Kidney'	*	NLL	NLL	NLL, SN
Glycine max				
'Bragg'	—	—	NLL, VN, D	NLL, VN, D
'Monetta'	—	NLL, VC	NLL, VC	NLL, VC
Vigna unguiculata				
'Early Ramshorn'	—	CLL	CLL, M	CLL
Cassia occidentalis	M	M	M	M
C. obtusifolia	NLL	NLL	NLL	NLL
Lupinus albus	M	M	M	M
Cyamopsis tetragonoloba	CLL, VC	CLL, VC	CLL, VC	CLL, VC
Sesamum indicum	M	M	M	M
Nicotiana benthomiana	M, P	M, P	M, P	M, P
N. clevelandii	CS, M	CS, M	CS, M	CS, M
Chenopodium amaranticolor	CLL	CLL	CLL	CLL
C. quinoa	CLL	CLL	CLL	CLL

[a] Plants sap-inoculated with infected tissue ground in 0.05 M phosphate buffer, pH 7.0, containing 0.02 M 2-mercaptoethanol and maintained under screen house conditions.

[b] CLL = Chlorotic local lesions, CS indistinct chlorotic spots,
D = distortion, M mosaic,
NLL = necrotic local lesions, P puckering,
SM = systemic mosaic, SN systemic necrosis,
VC = vein clearing, VN veinal necrosis,
— = no virus infection * test not done

Table 2

Serological relationships of peanut green mosaic virus isolates with antisera to other potyviruses

Isolate[a]	Antiserum to[b]								
	PGMV	SMV	AzBMV	BICMV	PStV	GEV	PVY	PSBMV	TEV
Non-systemic (NS)	1.81[c]	1.76	0.86	0.48	0.96	0.41	0.53	0.48	0.39
Systemic mosaic (SM)	2.00	1.96	1.06	0.89	1.22	0.41	0.58	0.93	0.63
Systemic necrosis (SN)	1.52	1.30	0.94	0.37	0.77	0.51	0.85	0.45	0.34
Healthy	0.03	0.03	0.04	0.01	0.03	0.01	0.04	0.01	0.02
Type strain	2.00	1.82	—	1.48	1.39	—	—	1.67	0.74

[a] Leaf extract for each isolate was diluted to 10^{-2} based on the original weight of tissue.

[b] PGMV = Peanut green mosaic virus; SMV = Soybean mosaic virus; AzBMV = Adzuki bean mosaic virus; BICMV = Blackeye cowpea mosaic virus; PStV = Peanut stripe virus; GEV = Groundnut eye spot virus; PVY = Potato virus Y; PSBMV = Pea seed-borne mosaic virus; TEV = Tobacco etch virus.

[c] Readings taken at A410 nm. Each is an average value for three replications.

producing distinct symptoms on groundnut, and thus they represent biologically distinct strains of PGMV. However, they did not show differences in their coat protein mol. wt. and serological reactions confirming that strains of several potyviruses cannot be differentiated on the basis of serology using polyclonal antisera (HOLLINGS and BRUNT 1981 a, b). Properties such as host range, cross-protection, morphology of cytoplasmic inclusions, aminoacid composition of the capsid protein, serology and cDNA hybridization have been used by many workers (JONES and DIACHUN 1977, REDDICK and BARNETT 1983, MOGHAL and FRANCKI 1976, ABU-SAMAH and RANDLES 1981, BARNETT *et al.* 1987, RANDLES *et al.* 1980) to differentiate individual potyviruses and their strains. Nevertheless, serological methods utilizing affinity column purified polyclonal antibodies directed towards virus-specific N terminus of capsid protein and HPLC peptide profiling of the capsid protein (SHUKLA and WARD 1989) may reveal critical differences between PGMV and its strains.

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