THE OCCURRENCE OF PEANUT MOTTLE VIRUS IN INDIA

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Published with the approval of the Director, ICRISAT, as Journal Article No. 41.

We are thankful to Dr. M. C. Padma, Department of Genetics, Osmania University, Hyderabad, for providing the facilities to perform the hemagglutination tests.

ABSTRACT

A virus causing mottle, interveinal depression and upward rolling of peanut leaf margins was found in the Punjab State. The pathogen was identified as peanut mottle virus (PMV) on the basis of serology, host range, aphid transmission, and physical properties in crude sap. The occurrence of PMV has not been reported previously from India. A highly sensitive serological method, using hemagglutination, was used successfully to detect PMV antigens in crude extracts of peanut, soybean, and pea.

Plant Dis. Reptr. 62: 978-982.

Peanut mottle virus (PMV) was first reported from the U.S.A. (11) and has since been positively identified in Africa (4), Australia (2), Europe (15), Japan (10), Philippines (3), South America (9), and West Malaysia (6). PMV is often overlooked in the field due either to mildness or masking of the symptoms and lack of marked reduction in plant growth (11). PMV has not been reported from India. During 1977 disease surveys were conducted in the major peanut-growing areas of India. A disease characterized by mottling of the new leaves and interveinal depression was occasionally observed in field surveys for viral diseases in the Punjab State. A sap and aphid-transmissible virus was isolated from diseased peanut plants. The purpose of this paper is to report identification of this virus as PMV based on the symptoms, transmission, host range, properties in crude sap, and serological affinities of the virus.

MATERIALS AND METHODS

Plants showing mottling and interveinal depression were collected from the crop raised by farmers in Samrala district of the Punjab State. Extracts from leaves were mechanically inoculated on peanut and the disease was subsequently maintained in a screenhouse in Arachis hypogaea 'TMV-2' and Glycine max 'Bragg' by mechanical sap inoculation.

All mechanical inoculations were made on carborundum-dusted plants with a cotton pad dipped in sap prepared with 0.05 M phosphate buffer, pH 7.0, containing 0.02 M 2-mercapto-ethanol (PBM). In host range studies at least six plants of each species were inoculated and maintained in a screenhouse at temperatures ranging from 15 to 25° C. Depending upon the symptoms produced, extracts from inoculated plants were assayed on Phaseolus vulgaris 'Topcrop' 3-8 weeks after inoculation.

Aphis craccivora colonies, maintained on Cajanus cajan (pigeonpea) and tested on healthy peanut plants, were used for transmission. All aphids were starved for about an hour and then allowed to make a single probe of 30 seconds to 2 minutes on young infected peanut leaves and then transferred to peanut seedlings. Exposed insects were left on the test plants for about 14 hr before they were killed by spraying with 0.1% S-[2-(ethylsulfinyl)ethyl] O, O-dimethyl phosphorothioate (active ingredient, oxydemeton-methyl) (Metasystox, Bayer (India) Ltd.).

Seeds from peanut cultivar TMV-2, showing well developed symptoms after being artificially inoculated, were tested for seed transmission by growing them in a screenhouse. Seedlings suspected of being diseased were tested for virus by assaying on Topcrop beans.

Tests of the properties of the virus in crude sap were conducted on the extracts from peanut and soybean prepared in PBM. For determining thermal inactivation point, 1 ml of the sap in 5.0-ml Corning tubes, diluted to 10^{-1} in PBM, was heated in a hot water bath for 10 minutes at various temperatures.

Each infectivity assay was performed on at least eight half leaves of Topcrop beans using an incomplete randomized block design.

Antiserum used for serological diagnosis was prepared against the N-strain of the virus as previously described (12). The agar gel double diffusion technique employed was similar to that described for potyviruses (8).

The hemagglutination technique employed was essentially similar to that described earlier (14). Tanned sheep red blood cells at 2.5% concentration were coated with antibody solution, diluted to 1/10 in 0.85% NaCl, at 37° C for 30 minutes. Excess antibody in the supernatant was discarded after low speed centrifugation and the cells were washed twice in 0.15 M phosphate buffered saline, pH 7.2 (PBS), containing 1/100 normal rabbit serum (ns PBS) and resuspended in the same solution to give a concentration of 2.5% packed cells. Extracts from leaves of infected peanut, soybean, pea plants, and comparable uninoculated plants, were prepared in PBS, pH 7.2, and clarified in a Beckman R-40 rotor at 3000 rpm for 10 minutes at 4° C. Serial two-fold dilutions of the supernatants were prepared in ns PBS and 0.5 ml was distributed to each of several wells in a plate. Then 0.08 ml of sensitized cells were added to each well and gently mixed. The plate was left at room temperature for about an hour and then incubated overnight at 5° C. A positive hemagglutination reaction consisted of a definite matting of cells with irregularity of the peripheral ring; in the case of a negative reaction red cells formed a perfect dense ring with a central pale disc.

RESULTS

The new leaves of mechanically-inoculated peanut plants showed vein clearing and mild mottling (Fig. 1). Subsequently, the leaf margins showed upward curling and interveinal depressions. As the leaves aged the symptoms became masked, but occasionally dark green islands were visible. The newly unfolded leaves of plants 2 months and older sometimes showed typical symptoms of the disease.

For testing the host range only selected species were included. Topcrop beans developed reddish brown necrotic lesions in 5 to 8 days after inoculation. Although a few lesions tended to spread along the veins and produced necrosis, the infections remained localized (Fig. 2). On bean cultivar Prince, veins of inoculated leaves showed necrotic streaking but the symptoms did not become systemic. On Cassia obtusifolia, inoculated leaves produced black necrotic spots and the leaves developing subsequently showed a systemic mottling. On Pisum sativum 'Bonneville', Glycine max Bragg (Fig. 3), and Vigna unguiculata 'California Black Eye', vein clearing followed by systemic mottle symptoms was observed.

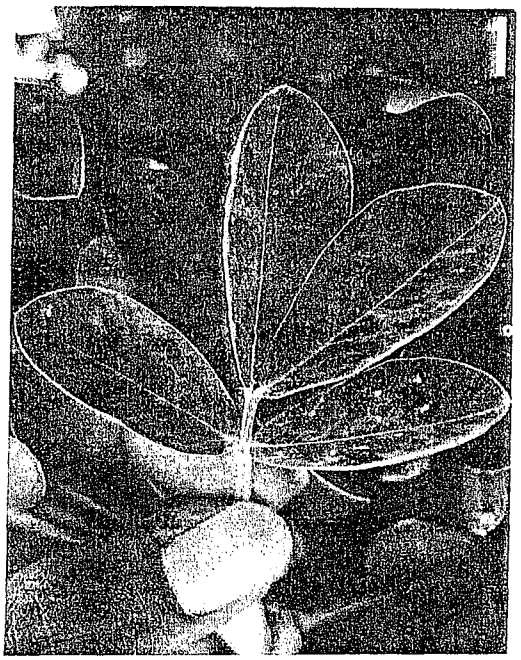
The virus could not be isolated from symptomless plants of the following species: Chenopodium amaranticolor, C. quinoa, Datura stramonium, Gomphrena globosa, Lycopersicon esculentum 'Pusa Ruby' and 'Marglobe', Nicotiana rustica, N. glutinosa, Petunia hybrida, and Vinca rosea.

The virus in sap, diluted to 1/10 in PBM, remained active for 48 hr at 25° C but not for 72 hr. Extracts prepared in PBM were infective at a dilution of 10^{-3} but not 10^{-4}. The virus had a thermal inactivation point between 55° and 60° C.

Of the peanut seed collected from infected plants, 310 germinated and four plants were infected giving a seed transmission frequency of 1.3%.

After short acquisition probes Aphis craccivora transmitted the virus from peanut to peanut. An acquisition feeding period of 30 seconds was adequate to transmit the virus.

In agar gel double diffusion tests, sap extracts from infected peanut leaves reacted with PMV-N antiserum diluted 1/10 but not 1/20. Extracts from healthy leaves formed no precipitin lines with concentrated antiserum.



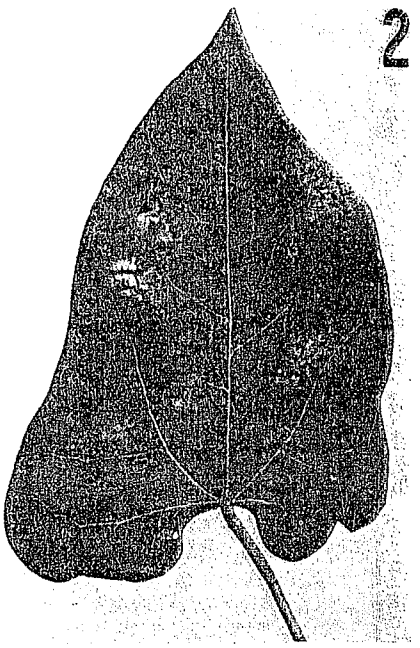
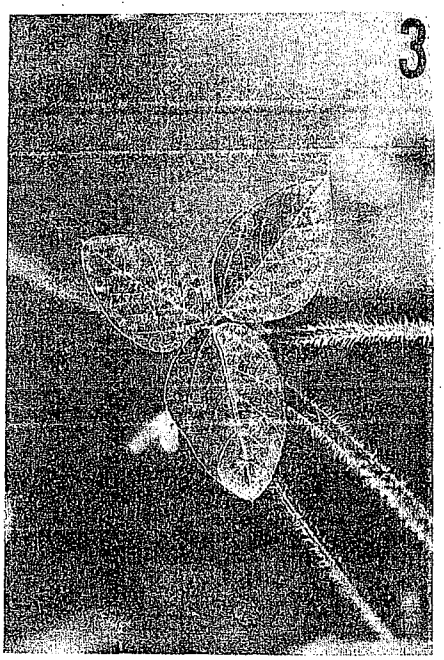


FIGURE 1. Mosaic symptoms produced by peanut mottle virus on peanut cultivar TMV 2.

FIGURE 2. Necrotic local lesions and veinal necrosis caused by peanut mottle virus on 'Topcrop' beans.

FIGURE 3. Mosaic symptoms induced by peanut mottle virus on 'Bragg' soybean.



In hemagglutination tests, extracts from healthy and infected leaves from peanut, soybean, and pea were reacted with PMV, tobacco mosaic virus (TMV), and soybean mosaic virus (SMV) antisera. Positive and negative reactions obtained with extracts from soybean plants are illustrated (Fig. 4). It was not possible to test dilutions of 1/10, 1/20, and 1/40 as they lysed the red blood cells. Extracts from healthy plants tested with all antisera and extracts from infected leaves tested with TMV and SMV antisera gave negative reactions. The serological dilution endpoints of PMV from infected peanut, soybean, and pea were 1/800, 1/1600, and 1/6400, respectively. Results of the hemagglutination test were similar in six experiments.

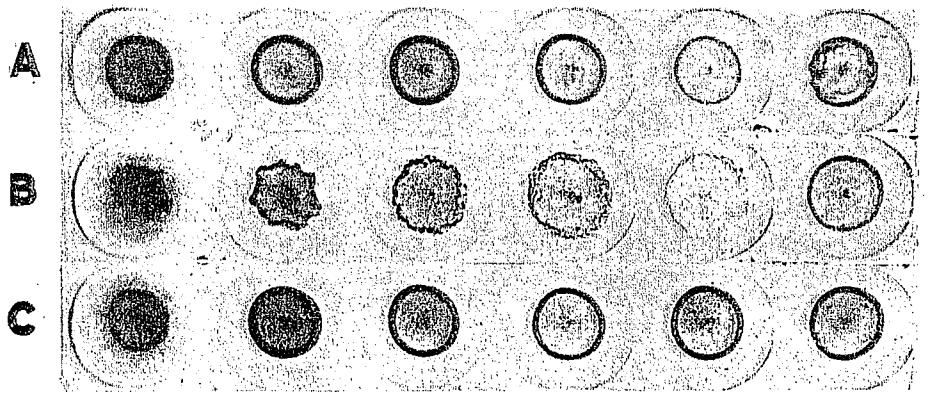


FIGURE 4. Hemagglutination results of healthy and peanut mottle virus-infected soybean extracts, after 16 hr of incubation (see text for details). Each horizontal row contains serial two-fold dilutions of extracts starting from the left, at 1/50 dilution. A -- Red blood cells were coated with peanut mottle virus antibody and tested with healthy soybean leaf extracts. Note the perfect dense ring of red cells characteristic of a negative reaction. B -- Blood cells coated with peanut mottle virus antibody were tested with peanut mottle virus-infected soybean leaf extracts. Note agglutinated red blood cells causing irregularity of the peripheral ring. C -- Blood cells coated with soybean mosaic virus antibody were tested with peanut mottle virus-infected soybean leaf extracts. Note negative results.

DISCUSSION

On the basis of serology, symptoms on peanut, host range and reaction, and aphid transmission, the virus found in peanut was identified as PMV. This paper presents the first record of its occurrence in India. There are no significant differences in the symptoms produced on peanut and other susceptible hosts from those reported for PMV from different countries (2, 4, 9, 11). In physical properties the PMV strain occurring in India bears a closer resemblance to the strain reported from Africa (4). Although the number of samples employed for seed transmission is inadequate for a critical evaluation of seed transmission, the results agree with those obtained with the mild mottle isolates (1).

For the first time it has been shown that the hemagglutination technique can be applied to detect PMV antigen. Virus titers are at least 80-fold higher than those obtained with agar double diffusion. Because the test is highly sensitive, it is likely that it has the potential to differentiate homologous and heterologous reactions between different PMV strains. In addition, it should be possible to investigate serological relationships among several potyviruses employing hemagglutination.

Several virus diseases have been reported on peanut from India (5, 13) but excepting tomato spotted wilt virus (7), they have not been thoroughly characterized for adequate comparison with PMV. Experiments on the purification of PMV and extensive surveys for the precise distribution of PMV in India have been initiated.

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