

Screening Peanut Germ Plasm Lines by Enzyme-Linked Immunosorbent Assay for Seed Transmission of Peanut Mottle Virus

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ABSTRACT

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Enzyme-linked immunosorbent assay (ELISA) was successfully employed for detecting peanut mottle virus (PMV) in peanut seed. Virus could be detected in extracts of infected cotyledons and embryos at dilutions of up to 1/3,600, although sensitivity was reduced when infected and healthy seed were mixed. PMV was detected in the testa of about 30% of infected seed. Complete correlation was observed among ELISA, infectivity assays, and growing-out tests for all four peanut cultivars tested. PMV was seed-transmitted in all but two—namely EC 76446 (292) and NC AC 17133 (RF)—of 283 peanut germ plasm lines screened.

Peanut mottle virus (PMV) is transmitted to a low proportion of peanut (*Arachis hypogaea*) seed (1,2,12). The importance of seed transmission in the epidemiology of PMV in peanuts has been established (4,5,8,10). Because of the low seed transmission rate and mild symptoms of PMV in peanuts, estimation of seed transmission percentage by symptom observation in progenies (ie, by the growing-out test) is unreliable. We have shown that enzyme-linked immunosorbent assay (ELISA) provides a more reliable and rapid method for routine testing for PMV in peanut seed. We also report the identification of two germ plasm lines in which no PMV seed transmission was detected in tests of more than 12,000 seeds.

MATERIALS AND METHODS

Virus culture. A PMV isolate found in India (12) was maintained in peas (*Pisum sativum* cv. Bonneville) by periodic mechanical inoculations with leaf extracts prepared in 0.05 M phosphate buffer, pH 7.0, that contained 0.02 M 2-mercaptoethanol (PBM).

Field inoculation. Infected pea leaves were ground in PBM (1 g/20 ml) and filtered through two layers of cheesecloth. Celite was added to a final concentration of 0.1%. Peanut plants at the fourth or fifth quadrifoliate stage were inoculated with an airbrush at an inoculation

pressure of 50 lb/sq. in. Leaves were immediately rinsed with tap water. Symptoms of PMV were scored 3 wk after inoculation.

ELISA. The ELISA procedures were similar to those of Lister (9) and Rajeshwari et al (11). Test tissues were ground in a mortar at a ratio of 1:10 (w/v) in antigen buffer (phosphate-buffered saline containing 0.05 Tween 20 and 2% polyvinyl pyrrolidone). Plates were coated with immunoglobulins precipitated from antiserum with 18% sodium sulfate and used at 1 µg/ml. Enzyme-conjugated immunoglobulins were used at 2 µg/ml. The enzyme used was alkaline phosphatase, with *p*-nitrophenyl phosphate as substrate. Results were scored after 30 min of substrate reaction time. Each tissue extract was tested in triplicate wells. Readings were considered positive if obvious yellow color developed or if the average absorbance ($A_{405\text{ nm}}$) value was at least twice that of healthy plant or seed-part extracts included as controls. Positive absorbance values ranged from about 0.4 to 2.0 after deducting values for healthy controls.

Infectivity assays. Leaf or seed-part extracts were ground in PBM and inoculated manually on the PMV local lesion host, *Phaseolus vulgaris* cv. Topcrop.

Growing-out tests. Seeds from PMV-infected plants were grown outside for symptom observation in an insect-proof screenhouse in containers of sterilized soil. Plants were sprayed with insecticide (*Metasystox*) at weekly intervals as a precaution against vector transmission.

RESULTS

Detection of PMV in various seed parts. Extracts from a small portion of the cotyledon from individual seeds of PMV-infected peanut plants (cultivar

Gangapuri) were mechanically inoculated on Topcrop beans, and only seeds that gave a positive assay were chosen for this study. Testas, cotyledons, and embryos were separated from 125 of these seeds, treated with trisodium phosphate followed by several rinses in distilled water, and tested by ELISA. The virus was detected in extracts from all cotyledons and embryos at dilutions ranging from 1/600 to 1/3,600. Amounts detected in embryo and cotyledon extracts from the same seed were the same; however, virus was detected in testas of only 43 of the 125 seeds tested and the titers in testa extracts never exceeded 1:300.

Correlation among growing-out tests, infectivity assays, and ELISA. Four peanut cultivars commonly grown in India were tested: TMV-2, Robut 33-1, Gangapuri, and M-13. Without destroying the embryo, small portions of cotyledons were dissected from each seed for ELISA. Preliminary tests showed that such treatment did not affect viability. Seeds were numbered and grown in trays in a screenhouse. Three weeks after germination, regardless of symptoms, each plant was tested by ELISA and infectivity assay (Table 1). There was complete correlation between the results of the two ELISA tests. Moreover, although at this stage not all the plants that ELISA indicated contained seed-transmitted PMV showed typical symptoms, they did so when grown for 3 wk more.

Detection of PMV in seed lots. The sensitivity of ELISA for detecting PMV in a large number of dry seeds was reduced by mixing infected and healthy seed. A small portion of an infected seed from the cultivar TMV-2 was added to 5, 10, 15, 30, 50, or 100 parts of seed from healthy TMV-2 plants and the mixture was ground in 5 ml of antigen buffer per gram. In five separate experiments, the virus was not detected when infected seed parts were mixed with 30 parts or more of healthy seed. This was true even when the infected seed contained high virus concentrations (titer of 1:3,600).

Frequency of seed transmission. Two hundred eighty-three peanut lines were inoculated in the field and seed from infected plants was collected. Seed transmission frequency was tested initially by grinding small portions of cotyledons from samples of five seeds and

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Table 1. Correlation among growing-out tests, infectivity assays, and enzyme-linked immunosorbent assay (ELISA) for detecting peanut mottle virus in seed of four peanut cultivars

Cultivar	No. of seeds tested	Positive by ELISA ^a (%)	Growing-out tests ^b		
			No. of seeds germinating	Positive by ELISA (%)	Positive by infectivity assays (%)
TMV-2	1,475	1.1	1,184 ^c	1.2	1.2
Robut 33-1	1,300	0.85	988 ^d	0.81	0.81
Gangapuri	1,150	1.6	955 ^c	1.8	1.8
M-13	950	1.4	646 ^c	1.4	1.4

^a A small portion of the cotyledon was tested retaining the embryo.

^b Each of the 3-wk-old germinated seedlings was tested by ELISA and by infectivity assays.

^c One seed that gave a positive ELISA did not germinate.

^d Three seeds that gave a positive ELISA did not germinate.

^e Four seeds that gave a positive ELISA did not germinate.

Table 2. Frequency of seed transmission of peanut mottle virus in 25 representative genotypes of the 283 tested by enzyme-linked immunosorbent assay (ELISA)

Genotype ^a	Growth habit ^b	No. of seeds tested ^c	Percent seed infection
EC 76446 (292)	EB	12,800 ^d	0.0
NC AC 17133 (RF)	SB	12,200 ^d	0.0
SAM Coll. (205)	EB	3,000	0.07
PI 259747	EB	2,200	0.09
NC AC 17788	SB	900	0.10
PI 261945	EB	828	0.12
RS-131	EB	675	0.15
NC AC 16046	EB	500	0.20
RMP 49/41	R	780	0.26
RS-81	SB	699	0.29
NC AC 583	EB	670	0.30
PI 298115	SB	500	0.40
NC AC 17118	EB	235	0.43
Dwarf mutant	EB	924	0.54
NC AC 17149	EB	253	0.79
AH 811-2	EB	502	1.00
NC AC 306	R	126	1.60
NC AC 1105	R	285	1.75
Small Japan	EB	350	2.00
NC AC 1824	EB	203	2.96
NC AC 16040	EB	450	3.10
EC 24425	EB	276	4.00
NC AC 17124	EB	145	4.10
NC AC 16016	EB	350	4.30
Coll. No. 677	EB	580	4.80

^a Each genotype was field-inoculated with PMV.

^b EB = erect bunch, SB = Spanish bunch, and R = runner.

^c Samples of five seeds each were initially tested in each ELISA well. For samples indexing positive, each individual seed was retested.

^d ELISA of 2,100 and 2,400 5-wk-old progeny seedlings from EC 76446 (292) and NC AC 17133 (RF) seeds, respectively, were also negative.

assaying them together in a single ELISA plate well. All five seeds of the samples giving a positive assay were then retested individually by ELISA to determine the number of seeds infected. By this procedure, we were initially able to test 470 seeds in each plate. PMV was seed-transmitted in all germ plasm lines tested, with the exception of EC 76446 (292) and

NC AC 17133 (RF) (Table 2). To test if the growing season influenced seed transmission in these two lines, plants grown during both the rainy (June–October) and postrainy (November–April) seasons were inoculated in the field. Totals of 12,800 seeds of EC 76446 (292) and 12,200 seeds of NC AC 17133 (RF) were collected from the PMV-infected plants and subjected to ELISA and growing-out tests. PMV was not detected in any of them. To check the validity of these results further, leaf samples from 2,100 and 2,400 5-wk-old seedlings grown from EC 76446 (292) and NC AC 17133 (RF) seeds, respectively, were individually tested by ELISA. None indexed positive for PMV (Table 2).

DISCUSSION

Results indicate that individual peanut seeds can be tested for PMV by ELISA without destroying seed viability. The virus was detected in all seed parts. In more than 60% of infected seed, however, the virus was not detected in testas although it was detectable in embryos and cotyledons. The virus concentration in cotyledons appeared to be about the same as in embryos. These results agree with those reported by Jafarpour et al (6) for bean common mosaic virus. Adams and Kuhn (1) reported, however, that PMV was absent in cotyledons and testas of seeds in which it was present in embryos. If the infectivity assay on Topcrop beans employed in their study was adequate to detect virus in cotyledons, there is a possibility that certain PMV isolates are restricted to the embryo.

The frequency of seed transmission observed in our work ranged from 0 to 4.8% (Table 2), which agrees with most previous reports (1,7,10). The high frequency of seed transmission of nearly 20% recorded for a PMV isolate in East Africa (3) seems exceptional and has not been demonstrated with PMV isolates occurring elsewhere.

Plants infected via seed have been shown to provide the primary source of PMV infection in the field (4,5), thus

PMV incidence could be reduced or eliminated by sowing virus-free seed. We were unable to detect seed transmission in more than 12,000 seeds each of EC 76446 (292) and NC AC 17133 (RF). Although these results do not eliminate the possibility of seed transmission at an extremely low frequency, they strongly suggest that these genotypes may be useful in breeding programs designed to produce cultivars with negligible seed transmission. Efforts are being made at ICRISAT to achieve this goal in crosses with high-yielding local cultivars. Also, EC 76446 (292) and NC AC 17133 (RF) are being tested for seed transmission of four PMV isolates from Georgia.

The potential of ELISA for detecting virus in a small fragment of the seed without affecting its viability will be useful in avoiding problems in sampling and growing-out logistics in plant quarantine. In addition, the test would be useful for screening large numbers of germ plasm sources for seedborne PMV. Although results indicate that only small batches of seed can be grouped for testing, test sensitivity is potentially greater and may be improved by future work. For example, in preliminary tests, inclusion of 0.3 M urea and 0.2% 2-mercaptoethanol in the extraction buffer enabled detection of one part of infected seed in 50 parts of total seed.

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