Testing Peanut Seeds for Peanut Stripe Virus[1]

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Abstract

Peanut seeds were tested by the enzyme-linked immunosorbent assay (ELISA) for peanut stripe virus without affecting their viability. A portion of cotyledon (0.02-0.05 g) was removed from the end of the seed opposite the radicle and triturated in an antigen buffer. Virus was frequently detected in extracts of cotyledons and embryos but seldom in the testa. Portions of one infected seed in ten could be detected. Correlations among ELISA, infectivity assays, and growing out tests were obtained. The ELISA technique has the advantage of detecting individual infected seed allowing the elimination of the infected ones. Identified infected seed can be used for preservation of virus cultures.

Key Words: *Arachis hypogaea*, ELISA, peanut stripe virus, seed assay, seed transmission.

Peanut strip virus (PStV) was first detected in 1982 in peanut (*Arachis hypogaea* L.) plants grown in Georgia (2) from seed received from China (3, 4). Surveys in 1983 confirmed the presence of PStV in other major peanut producing states in the U.S. The distribution of PStV in Georgia and other states appeared to be restricted to institutional plantings. Seed provides a vehicle for long distance dissemination of viruses. The subsequent infected seedlings can then provide the primary source of virus for local spread when suitable vectors are present. Seed-transmitted inoculum may, therefore, have provided the primary source of the virus in the U.S. Thus, seed transmission is regarded as an important factor in the dissemination of PStV. Detection and elimination of infected seeds are expected to reduce the chances of the establishment of PStV in commercial plantings. In 1984, enzyme-linked immunosorbent assay (ELISA) was successfully employed for detecting peanut mottle virus (PMV) in peanut seed (1, 6). This paper reports on the utilization of ELISA in detecting PStV in individual seed from contaminated seed lots.

Materials and Methods

The virus isolate used in this study was previously described (4). Infected seed lots were obtained by inoculating 20 plants in the 3rd true leaf stage of each of Argentine and Florunner peanut cultivars in the greenhouse. Inoculated plants were maintained until they reached maturity stage.

The initial ELISA system was that of Bharathan *et al.* (1). Tests with modified antigen buffer involved deleting certain additives or adding diethyldithiocarbamate (DIECA), ethylene diamine tetra acetic acid (EDTA) or urea. In each case of deletion or addition, the antigen buffer was formulated at pH 5, 7.2, and 9. Comparative tests were then done using samples from the same infected seed. Each test was repeated three times with at least two replications. ELISA plates were the 96 well Dynatech Immulon II (Dynatech Laboratories, Inc., 900 Slaters Lane, Alexandria, VA 22314) and readings were taken by a Dynatech II ELISA reader (Dynatech Laboratories, Inc., Alexandria, VA 22314) at 410 nm.

Twenty five Florunner seed, tested positive for PStV by ELISA, were separated into testa, cotyledon, and embryo and 0.02 g from each seed section was triturated in 0.5 mL antigen buffer and tested by ELISA. Ten additional Florunner seed that tested positive for PStV by ELISA were selected to be individually bioassayed on *Chenopodium amaranticolor* to compare local lesion numbers with the values obtained from the ELISA tests. An 0.04g sample was removed from the cotyledon of each seed and triturated in 0.5 mL of either 0.01 M Tris, -phosphate or -citrate buffer at pH 7.2 containing 0.01 M mercaptoethanol and 1% Celite.

Seed previously assayed positive for PStV infection by ELISA were used to determine the effectiveness of the test in pooled samples. One infected seed (or an 0.04g seed piece) was combined with 4, 9, 29, 49, or 99 healthy seed (or seed pieces) and triturated in 10x antigen buffer (w/v) and tested by ELISA.

A total of 498 Argentine seed (obtained from greenhouse grown infected plants) were individually numbered and tested for PStV by ELISA. After testing, seed were separately planted in trays in a greenhouse. When the seedlings were in the fifth true leaf stage, visual symptoms were recorded and individual seedlings were tested for the virus by ELISA.

In addition, 3780 seed obtained from field grown plants were individually tested by ELISA. These seed were from parents in which PStV was identified but the percentage infection of the parents was unknown. While 54 seed were identified by ELISA as having PStV, 930 seed gave ELISA values of 1.1 to 1.2 fold higher than those of the healthy controls. These 984 seed were discarded. Of the remaining 2,796 seed that did not give ELISA values above those of the healthy controls, 730 seed were planted in an isolated field in Spalding Co., Georgia that was never used for peanut production, and 1210 were planted in a greenhouse. The remaining seeds were planted in Tift Co., Georgia.

Two cultivars (Argentine and Florunner) of peanut were planted in the greenhouse at two different times so that maturity was reached during the winter or summer. The parent plants were mechanically inoculated with PStV in the 3 to 5 leaf stage. At maturity, seed were harvested and subsequently tested for seed transmission by sowing in steam sterilized soil in germinating trays in the greenhouse. Seedlings in the 5th to 6th leaf stage were individually tested using ELISA.

Results

Preliminary tests on 50 seed from greenhouse-grown plants using the ELISA system described earlier (1) revealed that 11 seed tested positive for PStV. These eleven seed were used as an antigen source for the comparative antigen buffer tests.

Antigen buffers with pH 7.0 gave higher readings (A_{410}) than those with a low (pH 5.0) or high (pH 9.0). Infected seed triturated in an antigen buffer composed of 0.8 M sodium chloride (NaCl), 0.5 M sodium phosphate (Na_2HPO_4/NaH_2PO_4), 0.025 M potassium chloride (KCl), 0.05% Tween 20, 0.1 M ethylene diamine tetra acedic acid (EDTA), and 2.0% polyvinylpyrrolidone (PVP) (40,000 MW) gave an average (6 tests) of 1.6 fold increase over the healthy controls. Average values from infected seed in the same buffer with 0.3 M urea gave a 2.1 fold increase. Average values with urea but without EDTA and PVP gave a 2.4 fold increase but gave a 2.5 fold increase without urea, EDTA, and PVP. Thus the antigen buffer for subsequent peanut seed test was composed of 0.8 M NaCl, 0.5 M PO_4, 0.025 M KCl, and 0.05% Tween 20.

PStV was consistently detected by ELISA in the cotyledonary and embryonic tissues of infected seed but not in the testa (Table 1). In this group of tests, ELISA values for cotyledonary and embryo tissue averaged re-

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spectively 2.5 and 2.0 fold higher than healthy controls. The maximum ELISA values obtained from the testa was 1.5 fold higher than those from healthy controls but out of the 25 seed tested, 5 seed gave readings less than 1.1 fold higher even though the cotyledon and embryo tissue from the same seed gave positive reactions. In all the 25 infected seed tested, positive reactions were obtained for both the cotyledon and the embryo.

Table 1. ELISA values obtained from different seed parts when testing for peanut stripe virus from infected peanut seed.

Test	ELISA Values[1,2]		
	Testa	Cotyledon	Embryo
1	1.3	2.7	2.1
2	1.0	2.1	1.9
3	1.1	2.7	2.0

[1] Expressed as fold increases over healthy controls.

[2] Average of three individual seeds tested.

The results using three different buffering systems (Tris, phosphate, or citrate) for bioassaying the virus from infected peanut seed to *C. amaranticolor* were variable with each buffer giving the greatest number of local lesions in a separate comparative test. Since phosphate gave the most lesions from seed with apparent low virus titer, it was chosen as the bioassay recovery buffer. In spite of that, all ten infected seed tested gave some local lesions when bioassayed on *C. amaranticolor*, seeds that had high ELISA values did not always give high local lesion numbers when used for bioassay tests. While one seed whose ELISA value was 1.5 fold increase above healthy control gave an average of 62 local lesions per *C. amaranticolor* leaf, another seed that had an ELISA value of 2.7 fold increase gave an average of only 22 lesions per leaf.

Results using infected seed pieces or infected whole seeds in pooled samples were variable. ELISA values from pooled samples at a 1 in 9 ratio varied from a 1.5 to 2.5 fold increase over healthy controls. Values from pooled samples at a 1 in 29 ratio varied from a 1.0 (same as healthy controls) to 2.0 fold increase. Results from pooled samples of 1 in 49 or 1 in 99 rarely gave values higher than healthy control.

When individual infected seed were tested by ELISA, some seeds gave high while others gave low ELISA values. Retesting the same seeds confirmed these results. When seeds with high ELISA values were mixed with healthy seeds at a ratio of 1 in 29, the virus was still detectable but when seeds with low ELISA values were pooled with healthy ones the virus was detectable at a ratio of 1 in 9 or less.

Of the 498 seed tested, 83 were identified by ELISA as being infected with PStV. A reaction was considered positive when absorbance at 410 nm was 1.3 fold higher than that of the healthy control seed in the same ELISA plate. After planting, 82 of the 83 seed showed symptoms of PStV and all 82 seedlings assayed positive in ELISA tests. The seed that was judged positive in the seed test but did not have virus in the seedling had an ELISA value of 1.5 fold higher than the healthy controls. Of the 415 seed that tested negative in the seed test, only one produced a seedling giving a positive reaction for PStV when tested by ELISA. That particular seed gave a reading 1.2 fold higher than that of the healthy control.

No PStV infected seedlings were found by ELISA in a field plot planted with 730 seed that tested negative (ELISA values no higher than healthy control) for PStV. However, in a parallel experiment in the greenhouse, only one out of 1210 seedlings tested positive by ELISA. This seedling, which did not show any PStV symptoms in the beginning, though still giving positive results in ELISA tests, expressed symptoms of a faint oakleaf pattern that is typical of the chronic infection stage but not the initial stripe symptoms (4).

Both tests showed that the cultivar Argentine, a spanish type, had a higher percentage of seed transmission reaching 37% for summer harvest and 18% for winter harvest, with an average of 28%. Florunner, as a runner type, had a 19% and 11% seed transmission (summer and winter harvest, respectively) with an average of 15%.

Discussion

Peanut seed, a large seeded legume, are soft enough in the dry state to permit removing (cutting) a small portion without shattering the seed. PStV can be detected efficiently in peanut seed by ELISA and this system is a rapid and reliable tool to detect infection of individual peanut seed or contamination of seed lots.

In all 25 infected seed tested, PStV could be readily detected in the embryo and cotyledonary tissue. The ELISA values were always highest from the cotyledonary tissue making this the tissue of choice for assaying peanut seed. This permits assaying for PStV without harming the germination of the seed.

If a seed is infected, PStV appears to be distributed throughout the cotyledons since repeated samples from an identified infected seed always produced positives in ELISA tests. Furthermore, the ELISA values of individual seeds varied but were relatively constant for the same seed. Seed with low ELISA values were always used when determining the efficiency of the test using pooled samples so that any contamination in unknown pooled samples could be detected. In this system, PStV could be detected in pooled samples of one infected in nine healthy seed but not in higher dilutions.

Bioassay on a local lesion host such as *C. amaranticolor* can be useful but generally the number of local lesions obtained were not in direct proportion to the ELISA values.

In a separate test using 10 seed identified as PStV infected, correlations existed between seed testing by ELISA, infectivity assays from the seed to local lesion assay hosts, and growing out tests. If a sample from a seed tested positive in the ELISA, a second sample used in mechanical inoculation of *C. amaranticolor* induced local lesions, and the remaining seed (when planted) resulted in an infected seedling. However, correlations were not always obtained for tests using pooled samples. Local lesions were seldom obtained on *C. amaranticolor* from inoculations with sap from 1 in 9 pooled samples.

Out of 498 seed tested by ELISA, 415 seed were judged to be virus free. After germinating the 415 apparently healthy seed, only one seed produced a seedling containing PStV as evidenced by the ELISA test. That particular seed had a value of 1.2 fold higher than the healthy controls but was below the 1.3 fold arbitrary reading assigned as the cut-off point for healthy versus infected. From a total of 1,940 seed that tested negative for PStV by ELISA and had values not higher than those of the healthy controls, 730 seed were planted directly in the field and the rest (1,210 seed) were planted in the greenhouse. None of the 730 seedlings grown in the field had PStV while one seedling from the 1210 seed planted in the greenhouse did have PStV. The fact that an apparently healthy seed, as evidenced by ELISA, may give rise to an infected seedling could be accounted for by one of the following possibilities: 1. Although care was taken in the trituration process, it is possible that a seed piece was not adequately triturated. 2. The virus titer was so low in the seed that the ELISA test failed to detect PStV. 3. Only cotyledon tissue can be removed from seeds that are to be germinated and for this it is possible that this seed had virus only in the embryo. ELISA technique can therefore be a useful tool for detecting PStV in peanut seeds. However, testing cotyledonary tissue may not be adequate to guarantee a seed or a seed lot being virus-free.

Testing seed lots of plant introduction entries and plant breeders lines, for the possible contamination with infected seeds may be an important step before they are introduced into the U.S. The results presented in this paper suggest that seed lots should first be tested for PStV by ELISA and the seeds judged virus free can then be planted in a greenhouse. At the 5th true leaf stage, seedlings should be individually tested for the virus and if free they can be grown and propagated in an isolated field before they are released.

The ability to detect individual infected seed without affecting its viability has the advantage of providing a means of preserving PStV in seed without the fear of having contamination with another virus(es) or losing aphid transmissibility which has often been experienced with some potyviruses after repeated mechanical transmission (5, 7).

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