Seed Transmission of Peanut Stripe Virus in Peanut

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ABSTRACT

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A direct antigen-coated, indirect enzyme-linked immunosorbent assay (ELISA-DAC) reliably detected peanut stripe virus (PStV) in peanut seed. PStV was detected in the cotyledons and embryo of all 90 infected seeds and in the testa of two seeds collected before harvest. The rate of PStV seed transmission was affected by peanut genotype and length of infection. Incidence of PStV seed transmission varied from less than 1 to 50% in the 935 peanut germ plasm lines tested. The majority had seed transmission rates ranging from 5 to 20%. Peanut cultivars and lines of the Spanish type showed higher seed transmission rates than those of other types. Several low seed transmission lines (less than 1% seed transmission) were obtained. PStV was detected in embryos of immature seeds at nearly the same percentage during pod development. Frequency of seed transmission varied according to the age of plants when they became infected. PStV transmission was higher in small than in large seeds.

Peanut stripe virus (PStV), formerly reported as peanut mild mottle virus (PMMV), is one of the most economically important peanut viruses in China (5,14,17,19). PStV is widely spread in peanut in China and some southeastern Asian countries and was recently reported to have been introduced into the United States and India through seed of peanut germ plasm (4,9,10,14).

Transmission of PStV through peanut seed may occur at relatively high rates. This provides a source of the virus that could lead to rapid spread of the virus by aphids. Infection early in the growing season may cause greater yield losses (6,9,13,17,18). Little is known about the mechanism or factors affecting seed transmission of PStV in peanut seed. Unfortunately, no resistance has been found in the germ plasm collections of *Arachis hypogaea* L. in China or Indonesia (10,16,20). Use of no or low seed transmission peanut varieties could be an effective approach for control of this virus. A number of different ELISA formats using polyclonal and monoclonal antibodies have been reported for detection of PStV (6,8). We have developed a direct antigen-coated, indirect enzyme-linked immunosorbent assay (ELISA-DAC) for detecting the virus in peanut seed (3). Some results on screening of peanut genotypes for seed transmission of PStV have been reported (15). In this study, the rate and transmission of PStV, location of PStV in peanut seed, and the effect of peanut genotype and age at infection on the rate of seed transmission were determined.

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MATERIALS AND METHODS

For ELISA-DAC, about 20 mg of a cotyledon was dissected from each seed and ground in coating buffer for testing as described by Cai et al (3). Results were assessed both by visual inspection and measurement of absorbance. Positive reactions showed colors from yellow orange to red orange which could be divided into three grades: +++ = strong positive (OD value greater than 1.5, P/N value [OD value of sample/OD value of healthy seed] greater than 3.0); ++ = medium positive (OD value 1.0–1.5, P/N 2–3); and + = weak positive (OD value 0.75–1.0, P/N 1.5–2.0). Antiserum to PStV was produced by Xu et al (17). Goat antirabbit FC immunoglobulin conjugated to horseradish peroxidase was produced by the Beijing Biological Product Institute. ELISA plates were produced by the Shaing-hai Plastic Products No. 3 factory. This assay is reliable and more sensitive than infectivity tests on *Chenopodium amaranticolor* Coste & Reyn. The virus could be detected in ELISA-DAC when an extract of an infected seed was diluted with buffer up to 1,500 times.

Location of PStV within seed. Seeds that tested positive for PStV by ELISA were separated into testae, cotyledons, and embryos and tested by ELISA-DAC to determine the location of the virus in infected seed.

Peanut genotype. Nine peanut cultivars and eight germ plasm lines, four with low and four with high seed transmission rates, were chosen to test the consistency of PStV seed transmission in different genotypes. Peanut cultivars and lines were tested for three to four successive years between 1984 and 1989. Seeds (100–200) from naturally or artificially infected plants of each cultivar or line were tested for seed transmission by the growing-out or ELISA-DAC test. Two high (about 10%) and two low (about 1%) seed transmission lines were used when examining variation of the virus in pods and seeds during their development. Pods (10–20) were collected at 15-day intervals after pod formation began from a random sample taken from plants of each line infected by inoculation of young seedlings. Pods were separated into outer shell, testae, cotyledons, and embryo and tested for PStV by ELISA-DAC.

Plant age at inoculation. The cultivar Huohua 1 was grown in a plastic net chamber for comparisons of PStV seed transmission with time of infection. Six groups of 45 peanut plants each were inoculated with PStV. The first group was inoculated shortly after emergence, and the remaining five groups were inoculated at 15-day intervals. Seeds from peanut plants infected at different times were harvested at maturity and tested by ELISA-DAC. Immature seeds also were tested by ELISA-DAC at intervals from the beginning of pod formation until maturity. Positive reactions of 50 seeds were confirmed by the growing-out test or infectivity assay. About 1,000 plants of this cultivar were also grown in the field as a source of naturally infected seed. Infected peanut plants were marked when infection was first observed. Seeds from these infected plants were harvested and tested by ELISA-DAC.

Environmental factors. The cultivar Fuhuasheng, a high seed transmission cultivar, was planted on three dates (the end of April, the end of May, and the end of June) in Wuchang to determine the effect of planting date on seed transmission. Plants of all planting dates were inoculated with PStV soon after emergence. Seeds of infected plants were harvested and tested by ELISA-DAC. The seeds from peanut plants naturally infected with PStV were collected both from plastic-mulched and unmulched commercial fields in the Beijing area from 1985 to 1989 and tested for virus.

Screening for low seed transmission. Peanut germ plasm lines (935) were screened in Wuhan in three successive years, 1987–1989, for PStV seed transmission. Genotypes infected with PStV were obtained from epidemic areas. About 60 seeds of each genotype were tested by growing-out tests in the greenhouse. Seeds were also planted in the fields and plants were naturally infected by PStV usually up to 100% 8–9 wk after planting. Genotypes that failed to show

any external PStV symptom in the growing-out tests were harvested and later tested by ELISA-DAC.

RESULTS

Location of PStV within seed. PStV was consistently detected by ELISA-DAC in the cotyledonary and embryonic tissues of 65 infected seeds but not in the testae. In this group of tests, ELISA values (P/N) of cotyledonary and embryonic tissue averaged three- and 2.8-fold higher, respectively, than healthy controls. The average of ELISA values (P/N) of testae was less than one. In another test with a group of 39 mature or immature infected seeds collected before harvest, PStV was detected by ELISA-DAC in both cotyledonary and embryonic tissues of 32 seeds, in the cotyledonary or embryonic tissues of five seeds, and in the testa of two seeds.

Peanut genotype. Incidence of PStV seed transmission varied among the 935 peanut germ plasm lines screened from 1987 to 1989. The majority (64%) of the 935 lines tested had seed transmission rates ranging from 5 to 20%. Eight percent of the lines tested had seed transmission over 20%. The highest seed transmission in a genotype was 50%. Seed transmission in 24 lines was less than 1%.

Peanut lines of the Spanish type had higher seed transmission rates than other types. During 1984–1986, the incidence of PStV seed transmission in four Spanish peanut cultivars ranged from 4.2 to 12.0% with an average of 9%, whereas five cultivars of Virginia and Virginia × Spanish hybrids ranged from 1.0 to 3.8% with an average of 2.6% seed transmission (Table 1). Seed transmission rates of four high seed transmission lines ranged from 12.9 to 32.4% in 1986, 3.9 to 27.6% in 1987, 7.1 to 21.6% in 1988, and 6.7 to 21.6% in 1989, with averages of 20.6, 16.0, 13.0, and 14.0%, respectively. Seed transmission rates of four low seed transmission lines ranged from 0.5 to 1.5% in 1986, 0 to 2.1% in 1987, 0 to 2.1% in 1988, and 1.4 to 1.9% in 1989. with averages of 1.0, 1.2, 1.2, and 1.6%, respectively. This shows the rate of PStV seed transmission is an inherited characteristic and occurs at two levels.

Presence of the virus in various parts of the pod and seed from early infected plants of two low (F357 and F358) and two high (F261 and F269) seed transmission genotypes was studied during development in 1988. Virus was detected in 12–50% of the pod shells 110 days after planting but in 45–81% of the pod shells at 125 days and generally declined at later test dates. Virus was detected in only one of 383 testa tested. The virus was usually detected in both the cotyledons and embryo of an infected seed. Virus incidence in the cotyledons and embryos varied with time after infection, similar to the variation of virus detected in the pod shells, but was consistently lower. Incidence of seed transmission in the four genotypes after harvest (7.0% for F261, 11.8% for F269, 2.4% for F357, and 0.9% for F358) was similar to the incidence of virus detected in embryos at the latter test times (Table 2).

Plant age at inoculation. Seed transmission of PStV was correlated with the age of the plant when inoculated; infection of young plants resulted in more seed transmission than did infection of old plants. Plants of the cultivar Huohua 1 (a high seed transmission genotype) that were infected during late flowering, pegging, or pod formation stages produced three seeds that tested positive for PStV, and all three were small, crinkled, and immature. These seeds probably resulted from late flowers. No seed transmission was found in the plants inoculated in the pod filling stage (Table 3).

The virus was detected in a high proportion of the developing seeds of Huohua 1 from the plants inoculated in the later three periods. Detection was usually possible about 20 days after inoculation and gradually decreased to zero before harvest. However, when plants were inoculated at the pod filling stage, incidence of virus detected in the seeds remained high until harvest (Table 4). Further tests indicated that the virus was associated with the testae but was not transmitted to the progeny seedlings.

The rates of PStV seed transmission from plants of the cultivar Huohua 1 naturally infected in the field at different ages were similar to those from plants of the greenhouse tests. The incidence of seed transmission from plants marked as infected on 31 May (seedling stage) and 24 June (flowering and pegging stage) was 10.4% (11/106) and 10% (12/120), respectively. However, incidence of seed transmission declined to 3.9% (7/130) and 1.1% (2/175) for plants marked on 3 July (pod formation stage) and 24 July (pod filling stage), respectively.

Environmental factors. Incidences of seed transmission in plants of the cultivar Fuchuashen infected with PStV planted at the end of April, the end of May, and the end of June were 21.5% (16/74), 14.9% (11/74), and 18.9% (14/74), respectively. The average temperatures of both air and soil at the flowering and pegging stage were 3–5 C lower for peanuts planted at the end of April than at the end of May or June in Wuchang. Peanuts planted at the end of April were in the flowering and pegging stage in June. The average PStV seed transmission rate in infected plants from plastic mulched and unmulched commercial fields in Beijing was 0.32% (ranged from 0.13 to 0.5%) and 0.26% (ranged from 0 to 0.6%), respectively, over 4 yr (1985–1988).

Size of seed. For the cultivars used in this study, shape and seed coat color of seeds infected with PStV were similar to those of uninfected seeds. However, PStV transmission rates were higher in small seeds than in large seeds. In three lots of seeds, the incidences of seed transmission in small seeds were 26.8%

Table 1. Percentage of seed transmission of peanut stripe virus in nine peanut cultivars

Cultivars[a]	Type of cultivar[b]	Naturally infected plants			Inoculated plants		Average
		1984	1985	1986	1985	1986	
Haihua 1	V	...	1.0	1.6	0.0	1.2	1.0
Zhengzhou 7432	V × S	...	0.5	0.5	2.5	4.9	2.1
Hua 37	V × S	...	2.1	5.8	1.5	1.2	2.7
Jiyou 2	V × S	...	0.0	2.2	2.2	8.8	3.3
Xuchou 68-4	V × S	4.1	1.1	4.5	2.7	6.7	3.8
Hua 28	S	2.5	1.8	8.2	...	4.2	4.2
Baisha 1016	S	8.3	1.8	7.8	20.2	...	9.5
Huohua 1	S	...	...	...	8.6	12.0	10.3
Fuhuasheng	S	10.4	7.5	7.5	11.8	13/21.6	12.0

[a] 100–200 seeds were tested for each transmission rate.
[b] V = Virginia, S = Spanish, and V × S = Spanish hybrid.

Table 2. Percentage of PStV detected by ELISA-DAC during the development of pods and seeds of early infected plants of four peanut genotypes

Days after planting for assay	Stage of pod development	Testa				Pod-Shell				Cotyledon				Embryo			
		F261	F269	F357	F358	F261	F269	F357	F358	F261	F269	F357	F358	F261	F269	F357	F358
110	Pod formation	0.0	0.0	0.0	0.0	33.3	12.5	50.0	23.1	8.3	12.5	0.0	0.0	8.3	12.5	0.0	0.0
125	Pod formation	0.0	0.0	0.0	0.0	63.6	45.5	81.8	54.5	9.5	9.5	0.0	0.0	9.5	9.5	0.0	0.0
140	Pod filling	0.0	0.0	0.0	0.0	10.0	10.0	0.0	0.0	11.2	5.6	0.0	0.0	5.6	5.6	0.0	0.0
155	Pod filling	0.0	0.0	0.0	0.0	10.0	30.0	0.0	40.0	0.0	20.0	15.0	0.0	0.0	10.0	20.0	0.0
170	Pod mature	0.0	5.0	0.0	0.0	20.0	30.0	10.0	0.0	20.0	10.0	0.0	5.0	15.0	15.0	0.0	0.0

(26/97), 21.9% (16/73), and 5.3% (11/209) with an average of 18%, compared with 8.2% (6/73), 15.5%, (9/58), and 0.8% (2/258) with an average of 8.2% in large seeds. Results of the test on plant age at inoculation indicated that incidence of PStV transmission in small seeds increased in plants inoculated when older.

Screening for low seed transmission genotypes. No line without any seed transmission was found among 935 peanut lines screened for 3 yr (1987–1989). Six lines showed PStV seed transmission of less than 1%: F88-158 (0.44%, 2/451), F88-313 (0.47%, 2/425), F88-456 (0.47% 3/159), F87-243 (0.81%, 4/493), F87-326 (0.88%, 4/454), and F87-358 (0.97%, 9/926). F87-358 is one of three lines in which seed transmission of peanut mottle virus was not detected (D. V. R. Reddy, *personal communication*).

Table 3. Seed transmission of peanut stripe virus from plants inoculated at different ages

Days after planting	Development stage[a]	Seeds tested (no.)	Seeds positive by ELISA-DAC (no.)	Transmission (%)
15	Seedling	114	5	4.4
30	Seedling	179	7	3.9
45	Flowering and pegging	184	3	1.6
60	Flowering and pegging	127	2	1.6
75	Pod formation	125	1	0.8
90	Pod filling	123	0	0.0

[a] Growth period of peanut cultivar Huohua 1 used in this test is 120 days.

Table 4. Variation in detection of peanut stripe virus from seeds before harvest of plants of Huohua 1 inoculated at different ages

Days after planting	Development stage	Seeds testing positive[a] (%) at days after planting				Seed transmission after harvest (%)
		83 days	94 days	104 days	117 days	
60	Late flowering and pegging	7.1 (1/14)[b]	18.1 (4/22)	5.6 (1/18)	0.0 (0/18)	1.6 (2/127)
75	Pod formation	44.4 (8/18)	73.3 (11/15)	23.5 (4/17)	0.0 (0/19)	0.8 (1/125)
90	Pod filling	...	...	68.4 (13/19)	55.5 (10/18)	0.0 (0/123)

[a] By ELISA-DAC.
[b] Numbers in parentheses are number infected/total tested.

DISCUSSION

ELISA-DAC was successfully employed for the detection of PStV and other peanut viruses without destroying seed viability (3,8). In our comparisons of methods for detection of PStV, ELISA-DAC results were closely correlated with those of the growing-out tests and infectivity assays.

The detection of PStV in the embryo and cotyledons of many seeds, in either the embryo or cotyledons of only a few seeds but not in the testae of mature seeds, agrees with the results of Demski and Warwick (6). However, in our study, virus was also detected in the testae of a few immature seeds.

Seed transmission of PStV varied from less than 1 to 50% among the 935 peanut lines with the majority of lines having rates between 5 and 20%. Peanut mottle virus, another important potyvirus which infects peanut, has lower seed transmission rates (0 to 4.8% among 283 peanut lines) (1). Peanut cultivars and lines of the Spanish type usually had more pronounced disease symptoms of PStV and showed higher seed transmission rates than other genotypes.

Some of the variation in the incidence of PStV in seeds during development could be attributable to the small sample sizes, but the trend was reproducible in cultivars of high or low seed transmission. We conclude that incidence of PStV in embryos was relatively constant during development in both low and high seed transmission lines, and incidence in embryos during seed development was similar to the seed transmission rates found after harvest. Thus, the virus probably infects only a portion of the embryos of infected plants during an early stage of seed formation, survives through the developmental stages of seed maturation, and is transmitted to progeny seedlings. The difference between low and high seed transmission lines is that the incidence of embryo infection early in seed development is higher in high seed transmission lines than in low seed transmission lines. This mechanism of seed transmission is different from that of soybean mosaic virus in soybean seed where virus may be inactivated in some seeds during seed maturation (2).

Experiments conducted in both the plastic net chamber and in the field indicate that early infection, before the peak of flowering, resulted in more seed transmission than did late infection. Because peanut is characterized by continued flowering until maturation of the crop, seed transmission of the virus can occur from late flowers even when plant infection occurs after the peak of flowering. Our results also confirmed that late infection usually resulted in infected seeds that are small and poorly developed.

Lines with no seed transmission have been reported for peanut mottle virus in peanut and for soybean mosaic virus in soybean (1,7); however, so far we have not found lines in which PStV is not seed transmitted. Research on the variation of seed transmission in low seed transmission lines in different years will be continued. An additional 12 lines with less than 1% seed transmission in the 1989 screening will be retested.

Seed transmission rates differ with specific virus strains (11). Symptom variants of PStV have been reported (5,12). It is thus important to determine the strains or symptom variants of this virus in China and their relationship with seed transmission.

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