Resistance to Peanut Stripe Virus in *Arachis* Germ Plasm

J. N. CULVER and J. L. SHERWOOD, Department of Plant Pathology, Oklahoma State University, Stillwater 74078-0285, and H. A. MELOUK, USDA-ARS, Stillwater, OK

ABSTRACT

Culver, J. N., Sherwood, J. L., and Melouk, H. A. 1987. Resistance to peanut stripe virus in *Arachis* germ plasm. Plant Disease 71:1080-1082.

Peanut accessions of the Arachis section, *A. diogoi* (PI 468141 and PI 468142), *A. helodes* (PI 468144), *Arachis* sp. (PI 468345 and PI 468169), and of the Rhizomatosae section (PI 468174, PI 468363, and PI 468366) were evaluated for resistance to peanut stripe virus (PStV). These entries and a susceptible cultivar (Argentine) were mechanically inoculated with PStV. Three to 4 wk after inoculation, both inoculated and subsequently formed leaves from each entry were tested for PStV infection. Symptomatology, local lesion assay on *Chenopodium amaranticolor*, enzyme-linked immunosorbent assay, and electron microscopy were used to evaluate susceptibility or resistance. This inoculation and testing sequence was repeated three times for each entry. All entries except PI 468169 and the susceptible cultivar Argentine were negative for virus infection. To our knowledge, this is the first report of resistance to PStV in *Arachis*.

Peanut stripe virus (PStV), a potyvirus, was first detected in the United States in Georgia in 1982 (2). It had apparently been introduced through germ plasm lines from the People's Republic of China (1). Since then, PStV has also been detected in Florida, North Carolina, Texas, Virginia, and Oklahoma (1,2).

Reports indicate that PStV infections have been confined to research plots (1); however, there is potential for the spread of PStV to areas of peanut cultivation. The spread of PStV is facilitated by high rates of transmission in seed, transmission by aphid vectors, and a host range that includes several common weeds that may serve as reservoirs for the virus (1,2). The potential for economic losses in peanut from PStV infection is unknown, although previous studies have indicated a yield loss in peanuts of up to 20% (1).

To date, there has been no report of resistance to PStV in any cultivar or germ plasm line of peanut. However, entries in the taxonomic sections of Arachis and Rhizomatosae have been shown to be resistant to peanut mottle virus (PMV), another potyvirus serologically distinct from PStV (2,5). Resistance to PStV in the section Arachis would be important because of its cross-compatibility with cultivated peanuts. On the basis of resistance to PMV, germ plasm lines of the Arachis and Rhizomatosae sections were selected for testing resistance to PStV. Reactions of selected entries of the Arachis and Rhizomatosae sections showing resistance to PStV are reported in this paper.

MATERIALS AND METHODS

Selected accessions were propagated vegetatively by removing stem sections with four or five fully expanded leaves. Ten to 15 stem sections from each entry were rooted in Hoagland's solution in

Journal Series Article 5127 of the Oklahoma Agricultural Experiment Station.

Accepted for publication 30 June 1987 (submitted for electronic processing).

test tubes (1 × 14 cm) placed in clear polyethylene chambers on a greenhouse bench (4). Once rooted, the stem cuttings were moved into 11-cm pots containing a 1:4 sand-soil mixture.

PStV was obtained from J. W. Demski, University of Georgia, Georgia Experiment Station, Experiment, and maintained in both *Lupinus albus* L. and peanut cultivar Argentine. Inoculum was prepared by grinding infected leaf tissue in 0.01 M phosphate buffer, pH 8. New growth on each of the test plants was dusted with 400-mesh corundum, and inoculum was applied to the leaf surfaces with small cheesecloth pads.

Symptoms were recorded 2–3 wk after inoculation. Both inoculated and subsequently formed leaves from each entry were removed and tested for PStV infection by enzyme-linked immunosorbent assay (ELISA) and local lesion assay. This inoculation and testing sequence was repeated three times for each test plant. During the final test, leaves were also removed for examination by electron microscopy.

Polyclonal and monoclonal antibodies to PStV were prepared as described previously (7). Microtiter plates for ELISA were coated with a 1/1,000 dilution of rabbit anti-PStV polyclonal antibody in 0.05 M sodium carbonate buffer, pH 9.6, for 2 hr at room temperature. Plates were then washed three times with phosphate-buffered saline with 0.05% Tween 20 (PBS-Tween), and a 1/10 and a 1/100 dilution of test peanut tissue ground in PBS-Tween with 2% polyvinyl pyrrolidone (PVP, mol wt 40,000) was added. After an overnight incubation at 4 C, plates were washed three times with PBS-Tween, and a 1/100 dilution of mouse anti-PStV monoclonal antibody in PBS-Tween was added and incubated for 2 hr at room temperature. Plates were again washed three times in PBS-Tween, and a 1/1,000 dilution of anti-mouse IgG alkaline phosphatase conjugate (Sigma, No. A-5781) in PBS-Tween was added and incubated for 2 hr at room temperature. Plates were washed a final three times in PBS-Tween, followed by the addition of alkaline phosphatase substrate (*p*-nitrophenylphosphate, 1 mg/ml) in 0.1 M glycine buffer with 1 mM of $MgCl_2$ and $ZnCl_2$ (Table 1), pH 10.4, and incubated for about 20 min. Absorbance at 405 nm was recorded by a BIO-TEK EIA plate reader (BIO-TEK Instruments, Inc., Burlington, VT). Tissue from the healthy peanut cultivar Tamnut was used as a negative control, and tissue from the PStV-infected peanut cultivar Argentine, as a positive control. The minimum threshold positive value was taken as three times the mean absorbance for two wells of the healthy control on that plate.

Local lesion assays were performed with foliar tissue from each test entry, ground in PBS-Tween with 2% PVP. The ground tissue was inoculated as described before onto several leaves of *Chenopodium amaranticolor* Coste & Reyn. (2). One to 2 wk after inoculation, plants were examined for local lesion development.

Electron microscopy was done by leaf-dip assay (3). Two microliters of a saline solution was placed on Formvar-coated electron microscope grids. A leaf from a test plant was cut perpendicular to the midvein, and the cut edge was allowed to make contact with the saline on the grid for 2 min. The grid was negatively stained with 1% uranyl acetate for 2 min and wicked dry with filter paper. Ten to 15 grid openings in each grid were examined to determine the presence or absence of PStV.

RESULTS AND DISCUSSION

The germ plasm entries selected were wild peanut lines from the USDA-ARS germ plasm collection at Stillwater, OK, that were maintained through vegetative propagation. Attempts to propagate several test plants from each entry varied in success because of the difficulty in rooting and maintaining wild peanuts. This left some entries with low numbers of test plants. However, plants with the same PI number are genetically similar and any further vegetative propagations from these PI numbers should reflect the results of this study. Thus, we have included entries with low test plant numbers in our results.

ELISA and local lesion assay provided the clearest evidence for resistance to PStV for each of the three inoculating and testing sequences performed on each germ plasm entry (Table 1). ELISA absorbance readings for positive reactions were between 0.30 and 2.00 optical density units. All reactions obtained from the healthy cultivar Tamnut control and for the negative reactions ranged from 0.00 to 0.06 optical density units. Results of local lesion assays compared directly to those of the ELISA. In electron microscopy, positive samples had rod-shaped particles of the same length and diameter as particles found in the PStV-infected cultivar Argentine control. Negative samples showed no such particles. Results of electron microscopy matched the results obtained

Table 1. Reactions of selected germ plasm accessions and one susceptible cultivar to inoculation with peanut stripe virus (PStV)

Entry	No. plants tested	No. inoculations	Reactions in tests for PStV[a]			
			ELISA[b]	Local lesion assays[c]	Electron microscopy[d]	Symptoms[e]
Resistant						
PI 468141	2	3	–	–	–	–
PI 468142	10	3	–	–	–	–
PI 468174	2	3	–	–	–	–
PI 468363	3	3	–	–	–	–
PI 468366	6	3	–	–	–	–
PI 468144	1	3	–	–	–	–
PI 468345	10	3	–	–	–	–
Susceptible						
PI 468169	5	3	+	+	+	–
Argentine	5	3	+	+	+	+

[a] Tests conducted 3–4 wk after each of the three inoculations except for electron microscopy (– = negative and + = positive for PStV).
[b] Reaction of an indirect double-antibody sandwich enzyme-linked immunosorbent assay.
[c] Assay done by mechanical inoculation on *Chenopodium amaranticolor*.
[d] Uranyl acetate negative-stained preparations of leaflets conducted 4 wk after third inoculation.
[e] Presence of mosaic and striping pattern typical of PStV infection.

Table 2. Indexing of *Arachis* germ plasm tested for resistance to peanut stripe virus (PStV)[a]

Entry	Taxonomic section	Origin (collectors)[b]	Resistance to PStV
PI 468141 (*Arachis diogoi* Hoehne)	Arachis	Brazil (GK 30001)	Yes
PI 468142 (*A. diogoi*)	Arachis	Brazil (GK 30005)	Yes
PI 468174 (*Arachis* sp.)	Rhizomatosae	Brazil (GKPSc 30131)	Yes
PI 468363 (*Arachis* sp.)	Rhizomatosae	Paraguay (GKPSc 30116)	Yes
PI 468366 (*Arachis* sp.)	Rhizomatosae	Paraguay (GKPSc 30119)	Yes
PI 468144 (*A. helodes* Mart. ex Hoehne)	Arachis	Brazil (GK 30029)	Yes
PI 468345 (*Arachis* sp.)	Arachis	Bolivia (GKSSc 30102)	Yes
PI 468169 (*Arachis* sp.)	Arachis	Brazil (GK 30037)	No

[a] As determined by independent confirmation in ELISA, local lesion assay, and electron microscopy (see Table 1).
[b] Collectors' initials: G = W. C. Gregory, K = A. Krupovickas, P = J. Pietratrelli, Sc = A. Schinini, and S = Simpson.

from ELISA and local lesion assay. Symptomatology of inoculated germ plasm lines could not be used to determine resistance or susceptibility because of a lack of clearly defined symptoms in the susceptible test plants.

Of the eight entries tested for resistance to mechanical inoculation with PStV, seven were consistently shown to be free of virus by ELISA, local lesion assay, and electron microscopy. The seven entries were PI 468141, PI 468142, PI 468144, and PI 468345 of the Arachis section and PI 468174, PI 468363, and PI 468366 of the Rhizomatosae section (Table 2). PI 468169 and the control cultivar Argentine always produced a positive reaction for the presence of a PStV infection.

Among the germ plasm lines shown to be resistant to PStV, five have also been shown to be resistant to PMV. These five are PI 468141, PI 468142, PI 468174, PI 468363, and PI 468366 (5). In addition, PI 468141 and PI 468142 have previously been shown to be resistant to early leaf spot, an economically important disease of peanut caused by *Cercospora arachidicola* Hori (4). The discovery of resistance to PStV in germ plasm lines of the Arachis section and the cross-compatibility of these lines with cultivated peanuts should provide a means for moving PStV resistance into cultivated peanuts. This is important because PStV is found not only in the United States and the People's Republic of China but also throughout Southeast Asia, where peanut is an important agricultural commodity (6). Hence, the movement of PStV resistance into cultivated peanut would provide a basis for future control of this disease.

LITERATURE CITED

1. Demski, J. W., and Lovell, G. R. 1985. Peanut stripe virus and the distribution of peanut seed. Plant Dis. 69:734-738.
2. Demski, J. W., Reddy, D. V. R., Sowell, G., Jr., and Bays, D. 1984. Peanut stripe virus—a new seed-borne potyvirus from China infecting groundnut (*Arachis hypogaea*). Ann. Appl. Biol. 105:495-501.
3. Hill, S. A. 1984. Methods in Plant Virology. Blackwell Scientific Publications, Palo Alto, CA.
4. Melouk, H. A., Banks, D. J., and Fanous, M. A. 1984. Assessment of resistance to *Cercospora arachidicola* in peanut genotypes in field plots. Plant Dis. 68:395-397.
5. Melouk, H. A., Sanborn, M. R., and Banks, D. J. 1984. Sources of resistance to peanut mottle virus in *Arachis* germ plasm. Plant Dis. 68:563-564.
6. Reddy, D. V. R. 1985. Peanut mottle and peanut stripe virus diseases in Thailand and the Philippines. Plant Dis. 69:1101.
7. Sherwood, J. L., Sanborn, M. R., and Keyser, G. C. 1987. Production of monoclonal antibodies to peanut mottle virus and their use in enzyme-linked immunosorbent assay and dot-immunobinding assay. Phytopathology. 71:1157-1160.