Antigenic Diversity Among Peanut Stripe Potyvirus (Family *Potyviridae*) Isolates

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ABSTRACT

Several geographically and biologically distinct isolates of peanut stripe *Potyvirus* (PStV) were tested against available polyclonal and monoclonal antibodies. In direct antigen-coated, enzyme-linked immunosorbent assay (DAC-ELISA), the polyclonal antisera prepared against the purified virus reacted with all the isolates tested. However, a monoclonal antibody, 3AB5, also prepared against the purified virus showed dramatic difference in its reactivity to the same isolates. Tested against blotch isolates from Georgia, Virginia, and Florida, 3AB5 reacted with two of 10 isolates. The reaction was either strongly positive or negative. Antibody 3AB5 did not react with a necrotic strain of PStV from Taiwan. Results indicate the existence of antigenic diversity among PStV isolates.

Key Words: Diagnosis, ELISA, isolate variation, serological detection.

Peanut stripe *Potyvirus* (PStV) is one of the more commonly found viruses infecting peanut (*Arachis hypogaea* L.) (Demski *et al.*, 1993; Mishra *et al.*, 1993). The virus is a yield-limiting factor in several parts of the world and has become endemic in most peanut-growing countries (Demski *et al.*, 1993; Mishra *et al.*, 1993). In addition to peanut, PStV also is reported to cause significant yield losses on soybean in Taiwan (Vetten *et al.*, 1992). Several PStV isolates have been described from different countries (Chang *et al.*, 1990; Wongkaew and Dollet, 1990; Demski *et al.*, 1993). Blotch strains of PStV have become well established in the peanut belt of the U.S., but they do not lead to significant yield reduction (Lynch *et al.*, 1988). Several necrotic strains of PStV were described from Georgia, Taiwan, and Thailand (Chang *et al.*, 1990; Wongkaew and Dollet, 1990; Sreenivasalu *et al.*, 1992).

Little information is available on the serological reactivity of the various PStV isolates described so far, though there are virus-specific poly- and monoclonal antisera available. Knowledge of serological reactivity would provide information on the antigenic diversity of the virus and help to improve the specificity and reliability of the existing ELISA-based detection of PStV. ELISA offers a practical and reliable means for routine and/or large-scale screening of peanut for PStV. In a preliminary study, a PStV-specific monoclonal antibody, 3AB5 (Culver *et al.*, 1989) reacted with a blotch isolate of PStV (Gunasinghe *et al.*, 1994) but did not react with a necrotic strain of PStV from Taiwan (Chang *et al.*, 1990; Pappu *et al.*, 1998). To discover the potential antigenic variability, we tested the reactivity of 3AB5 and polyclonal antisera with several geographically and biologically distinct PStV isolates.

Materials and Methods

Isolates. The PStV isolates used in this study were listed in Table 1. All the isolates were maintained on *Lupinus albus* L. or *Vigna unguiculata* L. after their initial isolation through single lesion transfers from naturally infected peanut.

Antisera. A commercial potyvirus detection kit (Agdia Inc., Elkhart, IN) was used following supplier's instructions. A polyclonal antiserum supplied by Brandt Cassidy, Noble Foundation, Ardmore, OK prepared against a purified viral antigen was used at 1:5000 dilution. The monoclonal antibody, 3AB5 (Culver *et al.*, 1989), prepared against a blotch isolate of PStV was used at 1:250, 1:500, and 1:1000 dilutions. The alkaline phosphatase labeled anti-rabbit IgG and anti-mouse IgG (Sigma Chemical Co., St.Louis, MO) were used at 1:20,000 and 1:8000 dilutions, respectively.

Enzyme-Linked Immunosorbent Assay (ELISA). Direct antigen-coated plate ELISA (DAC-ELISA) (Clark and Bar-Joseph, 1984) was used to test the reactivities of the above antisera against various PStV isolates. One hundred microliters of each of the sap extracts prepared by grinding leaf tissue in 10 volumes (w/v) of sample extraction buffer (coating buffer containing 2% polyvinylpyrrolidone) were loaded into duplicate wells of ELISA plates (Nunc-Immuno™ plate, Naige Nunc Int., Denmark). Sap extracted from healthy tissue as well as buffer alone were used as negative controls. After loading the antigens, the plates were incubated at room temperature inside a humid box for 1 hr. Plates were washed 3¥ with PBS-Tween. A volume of 100 µL each of the appropriately diluted antibody in ECI buffer (PBS-Tween containing 2% polyvinylpyrrolidone and 0.2% bovine serum albumin) were then added to each well. After 2 hr of incubation at room temperature, the

Table 1. List of PStV isolates used in the study.

Isolate designation	State/country of origin	Reference supplied by
PStV-GA1	Georgia	J.W. Demski
PStV-GA2	Georgia	J.W. Demski
PStV-GA3	Georgia	J.W. Demski
PStV-GA4	Georgia	J.W. Demski
PStV-GA5	Georgia	J.W. Demski
PStV-GA6	Georgia	J.W. Demski
PStV-FL	Florida	D.E. Purcifull
PStV-B	Georgia	Gunasinghe *et al.* (1994)
PStV-VA	Virginia	Rechcigl *et al.* (1989)
PStV-Ts	Taiwan	Chang *et al.* (1990)

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plates were washed as before and 100 µL of diluted enzyme conjugate were added. The plates were washed after 1 hr of incubation at room temperature. After washing, p-nitrophenyl phosphate substrate (1mg/mL substrate buffer) was added at a volume of 100 µL per well. The reactions of the ELISA were read 1 hr after adding the substrate. The wavelength, 405 nm, was used with a Bio-Tek (ELX 808) ELISA reader (Bio-Tek Instruments Inc., Winooski, VT). Each serological test was repeated at least 3×.

Results and Discussion

The potyvirus group-specific antibody (Agdia Inc., Elkhart, IN) as well as the PStV-specific polyclonal antiserum reacted with the 10 isolates tested (Table 2). Antibody 3AB5 however, showed remarkable differences in its reaction with the isolates studied. While it reacted with two of the blotch isolates from Georgia (PStV-GA6 and PStV-B, Table 2), it did not react with the remaining blotch isolates. Similarly, 3AB5 did not react with the necrotic strain from Taiwan (PStV-Ts, Table 2). The reaction was either positive or negative and there was no gradient of color in the ELISA wells. The same results were obtained whether 3AB5 was used at 1:250, 1: 500 or 1:1000 dilutions.

Results with 3AB5 indicate antigenic diversity among PStV isolates. It is interesting to note that, although 3AB5 was prepared against a blotch isolate of PStV, it was unable to react with all the blotch isolates. This inability to react with certain blotch isolates could be due to the absence of 3AB5-specific epitopes on the virions of these nonreacting isolates. Similarly, the nonreactivity of 3AB5 with the necrotic strain from Taiwan also indicates the divergence in the epitopes in this isolate's coat protein (CP). Although the antibody 3AB5 was able to differentiate the necrotic strain (no reaction) from some of the blotch isolates, its use in differentiating biologically distinct isolates (necrotic versus blotch) may be limited since it failed to react with some of the blotch isolates (Table 2). Whether 3AB5 demonstrates similar differentiating ability against other known necrotic isolates is unknown.

Table 2. Direct antigen coated-ELISA reaction of poly- and monoclonal antibodies with various PStV isolates used in the study.[a]

Antigen	Potyvirus group-specific antiserum	PStV polyclonal antisera	PStV 3AB5 monoclonal antibody
PStV-GA1	++++	++++	-
PStV-GA2	++++	++++	-
PStV-GA3	+	++++	-
PStV-GA4	++	+	-
PStV-GA5	++++	++++	-
PStV-GA6	++++	++++	++++
PStV-FL	++++	+++	-
PStV-B	++++	++++	+++
PStV-VA	++++	++	-
PStV-Ts	++++	++++	-

[a]+ positive reaction, - negative reaction. The difference between minimum and maximum values of the ELISA reading (taken at 405 nm) divided by four was used to determine the range corresponding to one '+'. Values range to a maximum of four plus signs.

The nature and distribution of epitopes on PStV CP are not known. Considering the all or none reaction of 3AB5 with some isolates (Table 2), it is suspected that a single or group of amino acids contributes to this epitope whose absence results in the loss of reactivity. Culver *et al.* (1989) observed that 3AB5 did not react with a blotch isolate of PStV in Western blot assays. It has been shown that a single amino acid change can result in loss of reactivity of a citrus tristeza virus-specific monoclonal antibody (Pappu *et al.*, 1993). There are 17 amino acid differences between the CPs of necrotic strain PStV-Ts (3AB5 nonreacting) and the 3AB5-reacting blotch (PSTV-B) isolates. The majority of the differences are in the N-terminal region (Pappu *et al.*, 1998). These differences, either singly or in combination, may contribute to the discriminating nature of 3AB5. As more sequences of CPs become available, it may be possible to relate the sequence data and the epitope profiles with serological reactivities of these antisera.

Monoclonal antibodies detecting a broad range of biologically distinct potyviruses also have been reported (Mink and Silbernagel, 1992; Richter *et al.*, 1995). The monoclonal antibody (P-3-3H8) produced using an isolate of PStV was reported to cross react with many members of the *Potyviridae* including more than 50 virus species of the genus *Potyvirus* and several isolates of ryegrass mosaic virus (Richter *et al.*, 1995). Another monoclonal antibody (7C14) prepared against an isolate of PStV cross reacted with blackeye cowpea mosaic virus and peanut mild mottle virus (Culver *et al.*, 1989).

The existence of antigenic diversity in PStV suggests that choosing appropriate antisera that are specific enough to detect individual strains while at the same time retaining the broadest detection spectrum possible would be ideal for keeping the plant quarantine programs effective in identifying PStV infection. Using a mixture of poly- and monoclonal antibodies with different specificities may be needed to achieve this objective.

Acknowledgments

A.I. Bhat is supported by a visiting associateship (BOYSCAST) from the Dept. of Science and Technology, Government of India. S.S. Pappu is supported by a postdoctoral fellowship from the College of Agric. and Environ. Sci., Univ. of Georgia. We thank Dr. J.W. Demski, Dr. Brandt Cassidy, and Dr. D. E. Purcifull for providing PStV isolates; Dr. Brandt Cassidy for anti-PStV polyclonal serum; and Dr. John Sherwood for the 3AB5 monoclonal antibody.

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