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Use of Monoclonal Antibodies in Detection and Serological Classification of Peanut Stripe Virus[1]

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ABSTRACT

Monoclonal antibodies (MABs) to peanut stripe virus (PStV) were obtained by fusing spleen cells from mice immunized with PStV to a mouse myeloma cell line. Two IgG2a subclass MABs, each binding with different antigenic sites, and rabbit polyclonal antibodies (PABs) were compared for their reaction to PStV and other serologically related plant viruses. One MAB reacted to PStV but not to other serologically related viruses. The other MAB reaction of the MABs and PABs to PStV in peanut leaf and seed tissues were compared by enzyme-linked immunosorbent assay (ELISA), dot-immunobinding assay, and Western blotting. MABs were found to be equivalent to or better than PABs in detecting PStV by ELISA and dot-immunobinding assay, but one MAB was not suitable for Western blotting.

Key Words: Monoclonal antibodies, peanut stripe virus, serology.

Peanut stripe virus (PStV), a member of the potyvirus group, infects *Arachis hypogaea* L. and has been found throughout much of Southeast Asia, including the People's Republic of China, Thailand, and the Philippines (15). PStV was identified in the United States in 1982 and has since been found in most of the major peanut producing states, primarily in institutional plantings (5, 6). The spread of PStV in the United States has resulted in reduced germplasm exchange, destruction of field plots and seed in breeding programs, and delays in the release of new peanut cultivars (5).

Serological methods utilizing polyclonal antibodies (PABs) (5,8,16), and/or monoclonal antibodies (MABs) (3,4) have been shown to be useful in the detection of PStV in seed and foliar tissue. PABs produced to PStV also react to blackeye cowpea mosaic virus (BICMV) and soybean mosaic virus (SMV) (7). Such problems can generally be circumvented by the use of highly specific MABs for the serological detection of plant viruses (9,11). Yet the specificity of MABs in serological assays can be affected by treatment of the plant virus during the assay.

The purpose of this study was to compare the utility of a MAB with a high degree of specificity to PStV, a MAB reacting to PStV and other serologically related viruses, and PABs in serological assays for detection of PStV. The serological reactivity of the MABs was compared to PABs in enzyme-linked immunosorbent assay (ELISA), dot-immunobinding assay, and Western blotting for the detection of PStV in peanut leaf and seed tissues.

Materials and Methods

PStV source and purification.

A "blotch" isolate of PStV, obtained from J. W. Demski, Department of Plant Pathology, University of Georgia, Experiment, GA, was maintained in *Lupinus albus* L. Peanut mild mottle virus (PMMV), SMV, and BICMV were maintained in appropriate hosts.

PStV was purified from freshly cut infected *L. albus* as previously described (3,4,16). The virus concentration was determined based on an absorbance of 3.0 at 260nm being equivalent to 1 mg/mL.

Production and purification of polyclonal antibodies.

Polyclonal antibodies to PStV were prepared in a New Zealand white rabbit as previously described (16,17). The titer of the antiserum was 1024 in a microprecipitin test using purified PStV. The IgG fraction of the antiserum was obtained by precipitation with one volume of saturated ammonium sulphate (2), followed by ion-exchange chromatography with DEAE Trisacryl M (LKB, Gaithersburg, MD, 20877, U.S.A.). The IgG was conjugated to alkaline phosphatase according to Clark and Adams (2).

Monoclonal antibody production and characterization.

Mouse myeloma cell line p3x63Ag8.653 was obtained from E. L. Halk (Agrigenetics Corporation, Madison, WI 53716, U.S.A.) and maintained as described previously (17). Balb/c mice were immunized by intramuscular injection with 250-500 mg of purified PStV emulsified in an equal volume of Freund's complete adjuvant. Three injections were made 1 week apart, followed by an injection of 250 mg purified virus in distilled water 4 days before fusion.

The procedure used for cell fusion was adapted from Kohler and Milstein (12) with modifications described previously (17). Two weeks after fusion, hybridomas were screened for antibody production to PStV by ELISA. All ELISA incubation periods were for 2 h at room temperature unless otherwise stated. Microtiter plates were washed three times between each step with phosphate buffered saline containing 0.05% Tween-20 (PBS-Tween). ELISA was performed by coating microtiter plates with 1 mg/mL of the IgG fraction of the rabbit anti-PStV PAB in 0.05 M sodium carbonate buffer, pH 9.6 (coating buffer). PStV infected or healthy *L.*

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albus tissue (1 gm/10 mL), triturated in PBS with 2% polyvinylpyrrolidone (PVP, mol. wt. 40,000), was then added to the plates and incubated overnight at 4 C. Supernatant from each hybridoma cell line was added to both infected and uninfected control ELISA wells and incubated for 2 h. Anti-mouse alkaline phosphatase conjugate was used to detect hybridomas producing antibodies to PStV (17).

Hybridoma cell cultures positive in ELISA for infected tissue and negative for uninfected tissue were cloned using a soft agar method as described previously (17). The isotype of the antibody produced by cloned hybridoma cell lines was identified using an isotyping kit (Zymed Laboratories, Inc., San Francisco, CA 94080, U.S.A.). To determine if the MABs react to the same or different antigenic sites, an ELISA additivity assay was conducted according to Friguet *et al.* (10).

ELISA, do'-immunobinding assay, and Western blotting procedures.

Two ELISA formats were examined for detection of PStV in foliar and seed tissue. An indirect double antibody sandwich (DAS) ELISA using PAB as the capture antibody and MAB as the probe antibody followed by goat anti-mouse IgG alkaline phosphatase conjugate was compared to a direct DAS ELISA using PAB for the capture antibody and PAB conjugated to alkaline phosphatase as the probe antibody. An indirect nonsandwich (NS) ELISA using MAB as the probe antibody followed by goat anti-mouse alkaline phosphatase was compared to a direct NS ELISA using PAB conjugated to alkaline phosphatase. The NS ELISA was also used to compare the reaction of MAB 3AB5, MAB 7C14, and PAB to other plant viruses.

The dot-immunobinding assays was conducted as previously outlined (17). For Western blotting, samples from seed and foliar tissue were prepared and transferred to nitrocellulose as previously described (16). In Western blots with PAB, the procedure of Sherwood and Melouk (16) was used. In Western blots with MAB, the procedure used in the dot-immunobinding assay for detection of the viral proteins spotted on nitrocellulose was used.

Results

Reaction to other plant viruses. PAB, MAB 3AB5, and MAB 7C14 were tested for their serological reaction to PMMV, SMV, and BICMV by a NS ELISA (Table 1). These three viruses are reported to be serologically related to PStV (7).. Reactions were determined by comparing ELISA absorbance values of infected foliar host tissue to uninfected foliar host issues for each of the tested viruses. In a NS ELISA, the IgG fraction of the PAB conjugated to alkaline phosphatase reacted with PStV, PMMV, and BICMV. MAB 7C14 reacted with the same three viruses. MAB 3AB5 reacted to only PStV and PMMV. Because MAB 3AB5 did not react as well in the NS ELISA, an indirect DAS ELISA was also performed against the different plant viruses using MAB 3AB5. Results of the DAS ELISA were the same as those obtained in the indirect NS ELISA for each of the tested viruses. MAB 3AB5 only reacted to PStV and PMMV. MAB 3AB5 and MAB 7C14 reacted to different epitopes of PStV based on the additivity index. The index can range from 0 to 100%. Values greater than 60% indicate the MABs are binding at different sites (1,10). The MAB 3AB5 followed by MAB 7C14 resulted in an index of 63.3%, and the MAB 7C14 followed by MAB 3AB5 resulted in an index of 69.3%.

Table 1. Reaction of anti-PStV antibodies to different plant viruses in a non-sandwich ELISA.[1]

Virus	3AB5	7C14	PAB
Peanut stripe virus	+	+++	+++
Peanut mild mottle virus	++	+++	+++
Soybean mosaic virus	-	-	-
Blackeye cowpea mosaic virus	-	+++	++

1 +++, A405 = 0.6 to 2.0; ++, A405 = 0.1 to 0.6; +, A405 = 0.02 to 0.1; -, A405 < 0.02 after one hour incubation with substrate. Wells with healthy controls were adjusted to zero before reading experimental wells.

2 ELISA was performed on triturated virus-infected and healthy foliar tissue at a 1/50 dilution.

ELISA. ELISA formats with various combinations of PABs, MAB 3AB5, and MAB 7C14 were used for the detection of PStV in peanut leaf and cotyledonary seed tissues. MAB assays required the use of goat anti-mouse IgG alkaline phosphatase conjugate because serological activity of the MAB was lost when conjugated to alkaline phosphatase. This problem has been encountered when trying to conjugate other MABs to alkaline phosphatase (14,17). Comparisons between PAB conjugated to alkaline phosphatase and the indirect assays using MAB were made because earlier experiments showed that probes of PAB directly conjugated to alkaline phosphatase had less reaction to uninfected plant material than PAB followed by an anti-rabbit IgG alkaline phosphatase conjugate. The response time and background readings varied greatly in the different ELISA formats (Table 2). The formats with the quickest response and the lowest background readings for detecting PStV in peanut leaf tissue were two DAS ELISA formats using either the PAB alone or PAB in combination with MAB 3AB5 as the probe for PStV. MAB 7C14 in a NS ELISA also worked well for detecting PStV in peanut leaf tissue, although this modification required four times as long as the DAS ELISA formats. PStV was not as readily detected in infected peanut leaf tissue in a NS ELISA with conjugated PAB or with MAB 3AB5.

Table 2. Reaction of anti-PStV antibodies in two different ELISA formats for the detection of PStV in peanut leaf and cotyledonary seed tissues.[1]

	Double-Antibody Sandwich ELISA					
	3AB5 Time (min)		7C14 Time (min)		PAB Time (min)	
Sample	30	60	30	60	30	60
PStV-infected peanut leaf	1.40	2.00	0.23	0.38	2.00	2.00
Uninfected leaf	0.08	0.11	0.08	0.09	0.23	0.39
PStV-infested peanut seed[2]	0.76	1.30	0.85	1.40	1.70	2.00
Uninfected seed	0.13	0.20	0.13	0.22	0.01	0.02
	Non-Sandwich ELISA					
	3AB5 Time (min)		7C14 Time (min)		PAB Time (min)	
Sample	30	60	30	60	30	60
PStV-infected peanut leaf	0.03	0.05	0.32	0.52	0.12	0.70
Uninfected leaf	0.02	0.00	0.00	0.00	0.09	0.13
PStV-infested peanut seed	0.06	0.19	2.00	2.00	1.20	1.90
Uninfected seed	0.08	0.00	0.00	0.00	0.02	0.47

1 Average (A_{405}) of 3 ELISA wells taken 30 and 60 min post substrate addition.

2 Infested seed tissue obtained by mixing 50 µg/mL purified PStV with healthy triturated cotyledonary tissue.

MAB 7C14 in an indirect NS ELISA, or PAB in a direct DAS or NS ELISA, gave the most satisfactory results for the detection of PStV in spiked cotyledonary seed tissue (Table 2). All other formats resulted in either slow response times or high background levels.

Dot-immunobinding assay. PAB, MAB 3AB5, and MAB 7C14 were compared for the detection of PStV in a dot-immunobinding assay with purified PStV, PStV-infected peanut leaf tissue, and PStV infested cotyledonary seed tissue (Fig. 1). Virus within infected leaf tissue was detectable at a 1/1000 dilution only with MAB 7C14. Virus in infected cotyledonary seed tissue could be detected at a 1/1000 dilution with both MAB 3AB5 and MAB 7C14. MAB 3AB5 produced the lowest background response in the uninfected

seed tissue control. MAB 3AB5, MAB 7C14, and PAB detected purified PStV at a 1/1000 dilution of a 50 mg/mL viral suspension representing approximately 0.5 ng of PStV placed on the nitrocellulose. However, MAB 3AB5 responded less intensely than did MAB 7C14 or PAB for the detection of purified PStV. PStV-infected foliar or seed tissue and uninfected tissue could not be differentiated with PAB in the dot-immunobinding assay.

Western blots. In Western blots, MAB 7C14 and PAB could detect PStV in infected leaf and cotyledonary seed tissues (Fig. 2). Healthy tissue produced no reaction for either MAB 7C14 or the PABs. MAB 3AB5 could not detect PStV in either infected leaf or cotyledonary seed tissues using Western blotting (Fig. 2).

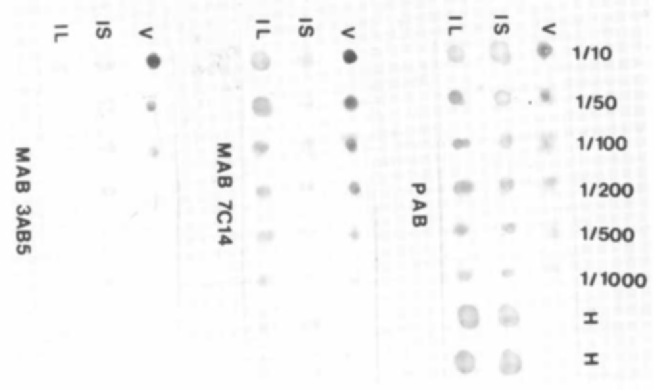

Fig. 1. Dot immunobinding assays with polyclonal antibodies (PAB), monoclonal antibody (MAB) 7C14, and MAB 3AB5. Dilutions were from 1/10 to 1/1000 of purified PStV (V), PStV infested cotyledonary seed tissue (IS), and PStV infected peanut leaf tissue (IL). Dilutions of healthy controls (H) for each sample MAB 7C14 and MAB 3AB5 indicating no reaction. For PAB they are purple in color indicating a false positive reaction. All other dots are purple in color.

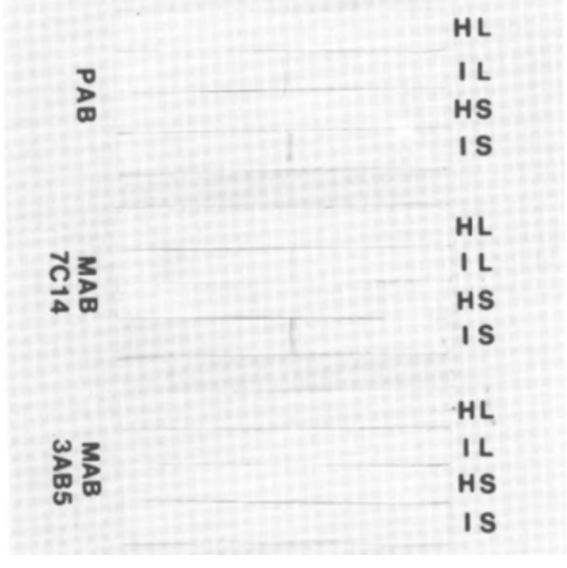

Fig. 2. Western blots using polyclonal antibodies (PAB), monoclonal antibody (MAB) 7C14, and MAB 3AB5. Lanes were loaded healthy peanut leaf tissue (HL), PStV infected peanut leaf tissue (IL), healthy cotyledonary tissue (HS), and PStV infested cotyledonary seed tissue. (IS).

Discussion

Serological cross reactivity has been demonstrated among PStV, BICMV, SMV and PMMV (6,7). The results from this study indicate that these shared epitopes may not always be expressed. The PStV PAB made in this study did react to BICMV and PMMV, but not to SMV. The MABs produced add some information to the serological relationship of these viruses. Both PStV and PMMV originated from China and have been considered to be isolates of the same virus (7,19). Both MABs reacted to PStV and PMMV indicating that the two viruses are serologically related. Since MAB 3AB5 only reacted to PStV and PMMV, these isolates have at least one unique epitope that is not common to the other serologically related viruses used in this study.

The DAS ELISA format, using only PAB, worked well for both peanut seed and leaf tissues. However, ELISA formats using MAB 3AB5 in an indirect DAS ELISA with leaf tissue, or MAB 7C14 in an indirect NS ELISA with seed tissue, worked equally well and gave lower background responses than that of the PABs.

The use of MABs in a dot-immunobinding assay provided the quickest and most convenient way for detecting PStV. This assay usually took about three hours to complete and was very reagent conservative. The use of PAB in the dot-immunobinding assay was unsuccessful for detecting PStV in leaf and seed tissue due to extremely high background responses of healthy tissue controls. This problem in using PAB in dot-immunobinding assays with peanut tissue has been previously reported (18).

Western blotting using MAB or PAB also provided a quick method for detecting PStV but requires more labor than either the dot-immunobinding assay or ELISA. The sensitivity of Western blotting is similar to that of ELISA in detecting PStV (16). Western blotting also has the added feature of separating samples by molecular weight. Molecular weight determinations made from Western blotting can be useful in detecting mixed infections of viruses in peanut (16).

The site that MAB 3AB5 reacts to must be altered after the virus is subjected to electrophoresis and blotting because MAB 3AB5 was not reactive in Western blotting but was in ELISA. This would suggest that the epitope is a result of the secondary or tertiary structure of the protein in the virion. The site that MAB 7C14 reacts to is not altered by the electrophoresis and blotting procedures since MAB 7C14 was reactive in Western blotting. This further supports additivity index data that the two MABs are reacting to different epitopes.

The recent parameters established for the identification of PStV (7) suggest PStV has a close serological relationship to BICMV and SMV. The results of this study with MABs indicate that PStV and PMMV have at least one epitope not shared with BICMV and SMV. The use of MABs in detection and identification of PStV may have considerable advantages over the use of conventionally prepared antiserum because of the high degree of specificity for PStV, but the assay of choice may affect the utility of MABs for detection of the virus.

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