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DIFFERENTIATION OF PEANUT CLUMP VIRUS SEROTYPES BY MONOCLONAL ANTIBODIES

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SUMMARY

A panel of monoclonal antibodies (mAb) produced against peanut clump virus (PCV) was used to characterize five serotypes of the virus. Four different formats of enzyme-linked immunosorbent assays (ELISA) were compared to establish the most suitable one for diagnosis of infected plants and for serotype differentiation. Since most mAb retained their activity when used for coating microtitre plates, a dual mAb-type assay was found to be most suitable. The same mAb could be used in ELISA as coating and as biotinylated antibody. Because of the ability of mAb to recognize subtle conformational alterations in the viral antigen, it is important to carefully select the ELISA format used for comparing different viral isolates.

KEY-WORDS: Peanut clump virus, Furovirus, ELISA; Monoclonal antibodies, Diagnosis, Phytopathology.

INTRODUCTION

Peanut clump virus (PCV), a member of the furovirus group (fungus-transmitted rod-shaped viruses), is an economically damaging pathogen occurring in West Africa, *e.g.* in Senegal (Bouhot, 1967), Burkina-Faso (Germani and Dhery, 1973), Ivory-Coast (Thouvenel *et al.*, 1976) and Niger. A similar virus, known as Indian peanut clump virus (IPCV), was described in India many years ago (Reddy *et al.*, 1979; Sundararaman, 1927).

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The natural vector of the virus is the fungus *Polymyxa graminis* (Dollet *et al.*, 1976; Thouvenel and Fauquet, 1980) but the virus can also be spread by seed transmission in groundnuts (Thouvenel *et al.*, 1978). In this host, it causes severe stunt and abnormally small dark green leaves.

In an attempt to characterize and differentiate PCV isolates from different countries, a panel of monoclonal antibodies (mAb) was raised to a PCV isolate from Senegal. In this paper, we report the production and characterization of these monoclonal antibodies and describe their use for differentiating five serotypes of the virus by enzyme-linked immunosorbent assay (ELISA). We also describe an ELISA diagnostic test suitable for detecting the virus in infected groundnut plants.

MATERIALS AND METHODS

Viruses.

Eleven PCV isolates obtained from infected peanuts were studied. PCV isolates K80, K84, K83 and K63 were collected in Senegal by J. Dubern and M. Dollet (Montpellier); isolates N2, N3 and N9 were from Niger and were obtained from D. Peters (Wageningen, The Netherlands). PCV isolates MJ and CHV from Burkina-Faso and S from Senegal were provided by J.C. Thouvenel (Adiopodoumé, Ivory Coast), while Indian peanut clump virus isolate Ludhiana was obtained from B.D. Harrison (Dundee, Scotland).

All isolates were mechanically transmitted first to *Chenopodium amaranticolor* (Coste and Reyn.) and subsequently to *Nicotiana benthamiana* (Domin.).

The virus was purified by the method of Thouvenel *et al.* (1976) with the following modifications: leaves of *N. benthamiana* were used unfrozen and after sucrose density gradient centrifugation, the virus-containing fractions were concentrated by ultracentrifugation.

For serological comparisons between PCV isolates, crude sap from infected plants was used. The leaf material was ground in phosphate-buffered saline (PBS) pH 7.4 (1 g leaves/10 ml buffer) and the plant sap was filtered through Miracloth.

Preparation of viral coat protein.

PCV coat protein was prepared from purified virus by the method developed by Durham (1972) for preparing coat protein of tobacco mosaic virus.

ACP = antigen-coated plate.
BSA = bovine serum albumin.
DAS = double antibody sandwich.
ELISA = enzyme-linked immunosorbent assay.
IPCV = Indian peanut clump virus.
mAb = monoclonal antibody.
PBS = phosphate-buffered saline.
PBST = phosphate-buffered saline with Tween.
PCV = peanut clump virus.

Polyclonal antibodies.

Rabbits received a series of injections of 200 µl of PCV K80 (0.5 mg/ml) emulsified in incomplete Freund's adjuvant at two-week intervals. The rabbits were bled 2 weeks after the third injection.

Hybridoma production.

Six BALB/c mice 6 weeks old were immunized by two intraperitoneal injections (100 µg of purified PCV K80 each) emulsified with an equal volume of complete Freund's adjuvant at 7-day intervals. One month after the last injection, the two mice giving the highest specific antibody response in indirect double sandwich ELISA were each given an intraperitoneal injection of 50 µg virus in saline. Three days after the second booster injection, spleens were excised and used for cell fusion. The fusion experiment was performed as described by Huss *et al.* (1987).

mAb were obtained in large amounts in ascitic fluids from pristane-primed BALB/c mice which had been injected with 0.5×10^7 hybridoma cells.

Immunoassays used for screening hybridomas.

Supernatants of growing hybridoma cultures were screened for the presence of specific antibodies to PCV K80 by an ELISA procedure 1 (double-antibody sandwich or DAS-ELISA) and procedure 2 (antigen-coated plastic or ACP-ELISA) using either purified virus or purified coat protein as antigen (table I). In procedure 1, the microtitre plates (Falcon 3911) were coated with 1 µg/ml anti-PCV rabbit immunoglobulin diluted in carbonate buffer pH 9.6. After 30-min incubation with 1 % BSA at 37°C, the plates were incubated with 1 µg/ml antigen in PBS containing 0.05 % Tween-20 (PBST). After washing with PBST, the plates were incubated with hybridoma culture supernatants (diluted 1/10) followed by alkaline-phosphatase-labelled sheep anti-mouse globulins (diluted 1/3000; Sigma, St. Louis). The enzyme conjugate was detected by addition of the substrate p-nitrophenyl-phosphate (1 mg/ml; Sigma) in 0.1 M diethanolamine buffer pH 9.8 at room temperature. In procedure 2, the plates were coated with 5 µg/ml antigen diluted in carbonate buffer pH 9.6. After saturating the remaining sites on the plastic with 1 % BSA, the

TABLE I. — **ELISA procedures used for hybridoma screening and detection of PCV isolates.**

Procedure	Successive steps of the assay			
1	Ab^R	Ag	mAb	anti-M^G-E
2	Ag	mAb	anti-M^G-E	
3	Ag	mAb-B	Stre-E	
4	Ab^R	Ag	mAb-B	Stre-E
5	mAb	Ag	Ab^R-E	
6	mAb	Ag	mAb-B	Stre-E

Ab^R = anti-PCV rabbit antibodies; Ag = antigen (purified virus, purified coat protein or crude sap from infected leaves); anti-M^G = goat anti-mouse globulins; E = enzyme label; B = biotin label; Stre = streptavidin.

subsequent steps of the assay were as in procedure 1 (table I). A buffer control without antigen was included with each tested supernatant. Furthermore, for each microtitre plate, a negative control with fresh culture medium and a positive control consisting of a mixture of culture supernatants containing antibodies released from unfused plasma cells were also tested.

A «Titertek Multiscan» photometer (Flow Laboratories) was used to measure the optical densities at 405 nm. Absorbance values were considered positive if they exceeded the buffer control by a factor of two.

Isotyping of mAb was done by ELISA procedure 2 using subclass-specific rabbit anti-mouse antisera (Nordic, Tilburg) and goat anti-rabbit alkaline phosphatase conjugate.

Biotinylation of mAb and preparation of enzyme-labelled streptavidin.

For biotinylation of mAb, N-hydroxysuccimidobiotin (Sigma) dissolved in distilled dimethylformamide was added to ascitic fluids (diluted 1/10 in 0.01 M $NaHCO_3$) in a 1/25 (v/v) ratio. The mixture was incubated for 4 h at 25°C and the reaction was stopped by addition of 1 M NH_4Cl (Zrein *et al.*, 1986).

A mixture of streptavidin (1 mg; Sigma) and 100 µl alkaline phosphatase (10 mg/ml; Boehringer) was first dialysed 5 h at room temperature against 0.1 M PBS containing 0.06 % glutaraldehyde (w/v) and then against several changes of PBS.

Immunoassays used for serotyping PCV isolates and for diagnosis.

Different ELISA procedures (table I) were evaluated for their ability to detect virus in crude sap from infected plants. These tests were performed with «Nunc Maxisorp F96» (Nunc, Roskilde) microtitre plates. The dilutions of mAb preparations used in these assays are indicated in table II. Assays have been described in

TABLE II. — **Reactivity of mAb with preparations of PCV and PCV protein in ELISA procedures 1 and 2.**

Type of antibody	mAb	Subclass type	Reactivity in ELISA procedure: 1 virus	1 prot.	2 virus	2 prot.	Working dilution of ascitic fluid: mAb used in procedures 1 and 2	coating (*) mAb used in procedures 5 and 6	biotinylated (*) mAb used in procedures 4 and 6
I	64.1	IgG2a	+	+	+	+	5×10^{-7}	10^{-5}	5×10^{-6}
	66.1	IgG2a	+	+	+	+	10^{-6}	10^{-4}	10^{-5}
	75.1	IgG2b	+	+	+	+	5×10^{-5}	10^{-3}	5×10^{-4}
II	17.2	IgG2a	+	+	+	−	10^{-5}	5×10^{-4}	5×10^{-5}
III	8.2	IgG1	+	−	+	+	5×10^{-5}	10^{-3}	10^{-5}
IV	44.2	IgG2a	−	+	+	+	5×10^{-6}	10^{-4}	5×10^{-5}
V	60.2	IgG2a	−	+	−	+	10^{-5}	10^{-3}	10^{-5}
	70.2	IgG2a	−	+	−	+	10^{-6}	10^{-4}	5×10^{-5}
VI	63.1	IgG2a	−	−	+	+	5×10^{-5}		

(*) These figures are given only for the purpose of comparison.

detail previously (Al Moudallal *et al.*, 1984; Zrein *et al.*, 1986). The streptavidin alkaline phosphatase conjugate and the anti-mouse globulin conjugate were used at a dilution of 1/2,000 and the rabbit antibody conjugated to alkaline phosphatase at a dilution of 1/1,000.

RESULTS

Fifteen stable clones secreting mAb specific for PCV were subcloned into 50 subclones. Most of them were injected into pristane-primed BALB/c mice to obtain ascitic fluids. When all positive hybridomas were tested in the form of culture supernatants against the different PCV isolates, it was observed that all subclones originating from the same initial clone had the same reactivity with all virus isolates. In subsequent tests, therefore, only one representative subclone was used. A total of 13 mAb were found to retain their reactivity when used for coating microtitre plates; nine of these mAb were selected for detailed study of their ability to bind to the different PCV isolates in various ELISA formats; these antibodies were also biotinylated.

ELISA procedures 2 and 3, which use antigen-coated plates (table I) were found to give high background values when crude plant extract from infected plants was used as antigen. Most comparisons, therefore, were made with ELISA procedures 1, 4, 5 and 6. The dilutions of ascitic fluids used in the tests were chosen in order to obtain an absorbance of 1.0 after 1-h substrate incubation and are shown in table II. A negative control consisting of plant sap from healthy plants was included with all assays performed with sap from virus-infected plants.

Characterization of monoclonal antibodies.

In order to make reliable comparisons of the ability of a mAb to recognize different virus isolates, it was important to know whether the reactivity of mAb depended on the form of the PCV antigen used in the assay. As shown in figure 1, it was found that the level of reactivity of the 9 mAb used in the study remained unchanged when they were tested by ELISA procedures 1 and 4 against the isolate K80 presented either as a crude sap extract or as a preparation of purified virus or purified coat protein. On the other hand, when tested by ELISA procedure 6, it was found that the mAb reacted to different degrees with isolate K80 depending on the type of antigen preparation used (fig. 2). The largest differences were observed with respect to the ability of the various mAb to act as trapping antibodies for the three types of antigen preparations (compare vertical columns); in contrast, biotinylated mAb (compare horizontal lines) gave more consistent results, especially when they reacted with crude sap or purified virus.

Certain combinations of coating and biotinylated antibodies gave rise to high reactivity with all three forms of the antigen (*e.g.* coating mAb

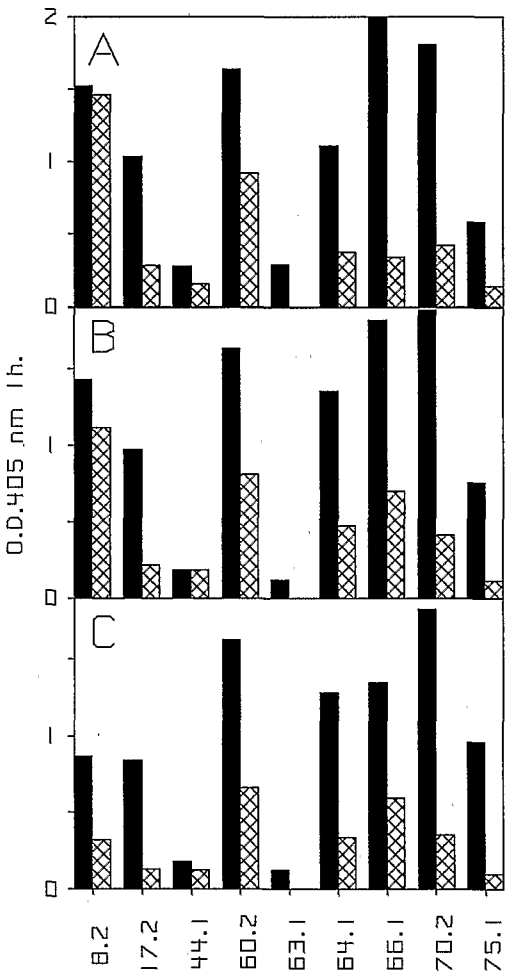


FIG. 1. — *Reactivity in ELISA procedures 1 and 4 of nine mAb to PCV with K80 antigen in the form of crude sap from infected plants (A), purified preparations of virus (B) and viral protein (C).*

Plates were coated with rabbit globulins to PCV K80. In ELISA procedure 1 (▬), the antigens were revealed by unlabelled mAb followed by a goat anti-mouse immunoglobulin conjugate. In ELISA procedure 4 (▨), the antigens were revealed with biotinylated mAb followed by labelled streptavidin. Ascitic fluids were diluted as indicated in table II. The substrate hydrolysis time was 1 h.

60.2/biotinylated mAb 66.1; coating mAb 66.1/biotinylated mAb 70.2; coating mAb 60.2/biotinylated mAb 17.2). These combinations of mAb are particularly suited for developing a diagnostic test relying only on the use of mAb. It is noteworthy that certain homologous combinations of the same mAb used both as coating and labelled antibody (mAb 17.2, 44.1 and 66.1) were highly successful when used for detecting virus particles. Although homologous combinations gave rise to a lower reactivity with purified viral

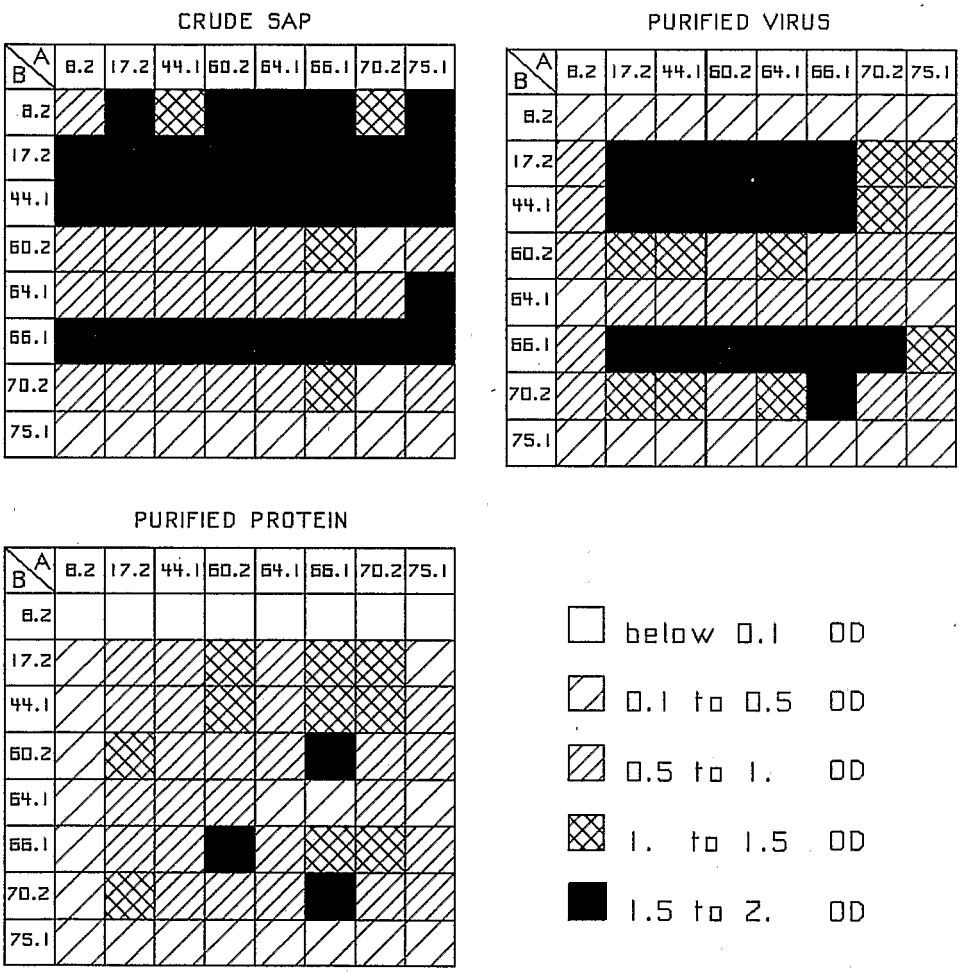


FIG. 2. — *Reactivity in ELISA procedure 6 of eight mAb to PCV with K80 antigen in crude sap and in purified preparations of virus and coat protein.*

Plates were coated with the mAb indicated in (A). The antigen was revealed by the second biotinylated mAb indicated in (B). Positive reactions are presented as black or hatched squares corresponding to absorbance values obtained after substrate hydrolysis. Ascitic fluids were diluted as indicated in table II.

coat protein than with virus, a significant level of reactivity (OD > 0.5) was observed in several cases. Since a viral subunit possesses only one epitope that can be recognized by any individual mAb, these results indicate that the coat protein preparation contains polymerized subunits presumably in the form of small oligomers.

The results observed with mAb 8.2 (coating and biotinylated antibody) and mAb 75.1 (coating antibody) (fig. 2) also demonstrate that the purification procedure alters the antigenic properties of the virus. Since electron microscope observations showed that the particles in purified virus preparations had an undamaged appearance (data not shown), these results are probably caused by some limited alteration of the epitopes recognized by mAb 8.2 and 75.1 respectively, possibly by proteolytic damage.

In view of the results presented in figure 2, all further comparisons between PCV isolates were carried out with infected *N. benthamiana* extracts (diluted 1/10) as antigen source.

Differentiation of PCV serotypes.

ELISA procedures 1, 4 and 5 were used to compare the reactivity of the 9 mAb with 11 PCV isolates (fig. 3). Four reactivity patterns could be distinguished. Isolates K80, K83, MJ and S showed the same pattern of reactivity in the three ELISA formats and were labelled serotype A; isolates N3 and N9 gave similar reactions with all mAb and were labelled serotype B; isolate K84 corresponding to serotype C was only recognized by mAb 66.1, which reacted strongly in procedure 1 and weakly in procedure 4; isolate K63 corresponding to serotype D was only recognized in procedure 1 by mAb 66.1. It should be noted that serotypes C and D were not recognized by anti-PCV K80 rabbit antiserum, which prevented them from being detected in ELISA procedure 5. Another point of interest revealed by a comparison of procedures 1 and 4 (fig. 3) is that biotinylation of the mAb always led to lower reactivity, except in the case of mAb 8.2, where unlabelled and labelled antibodies were equally active.

The results obtained when the PCV isolates were compared by ELISA procedure 6 are shown in figure 4. The pattern of reactivity shown by the four isolates of serotype A (K80, K83, MJ and S) and the two isolates of serotype B (N3 and N9) were again very similar. In contrast, serotypes C and D were more clearly differentiated than in figure 3. A fifth serotype E, corresponding to IPCV, was detected by biotinylated mAb 75.1, while isolates N2 and CHV were not detectable by any mAb (results not shown). It should be noted that only the 6 isolates corresponding to serotypes A and B were detected when the 11 PCV isolates were tested in DAS-ELISA using the anti-K80 rabbit globulins both as trapping and labelled antibodies. Somewhat unexpectedly, therefore, the use of mAb made it possible to detect three additional serotypes (C, D and E).

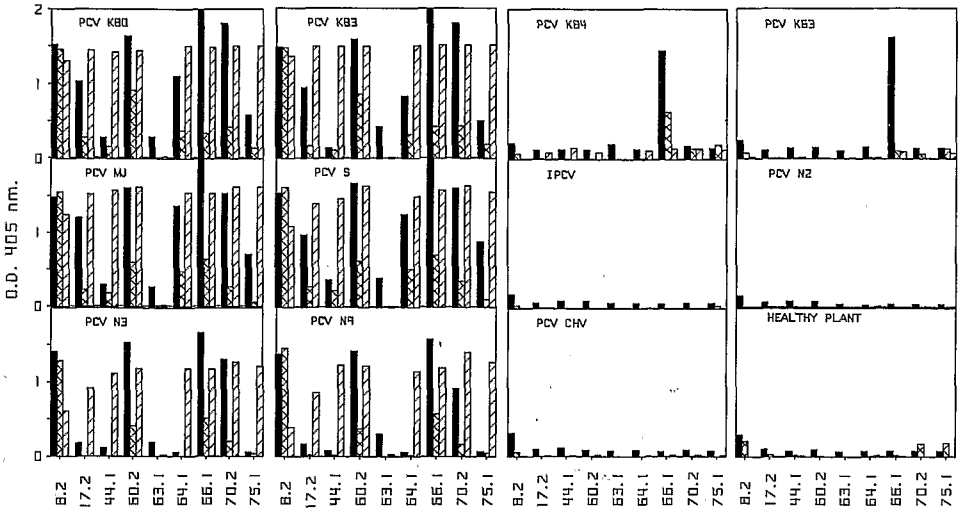


FIG. 3. — *Comparative reactivity of nine mAb tested against 11 PCV isolates in three ELISA procedures.*

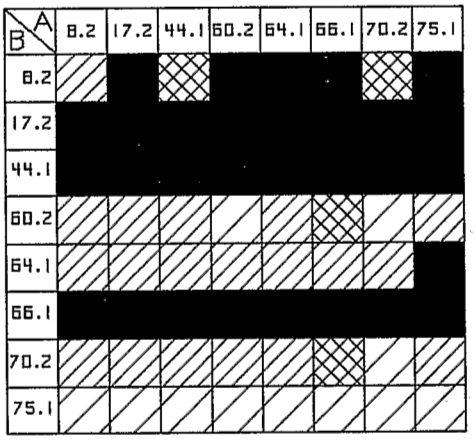
ELISA procedure 1 (▬) and 4 (⊠) were performed as described in figure 1. In ELISA procedure 5 (▨), plates were coated with mAb diluted as shown in table II. The antigen was in the form of crude extracts from infected plants.

The results obtained when the 11 PCV isolates were studied in the four ELISA procedures 1, 4, 5 and 6 are summarized in figure 5. In this figure, black squares indicate that the absorbance values in the test were greater than 0.5. The data obtained by procedure 6 correspond to a test in which the same mAb was used as coating and as biotinylated antibody. The results show that serotype A is recognized by the largest number of mAb, followed by serotype B, which is most frequently recognized in ELISA procedure 5. Serotypes C, D and E are only recognized by one mAb each and only in one or two ELISA formats. mAb 66.1 and 75.1 detected the largest number of PCV isolates.

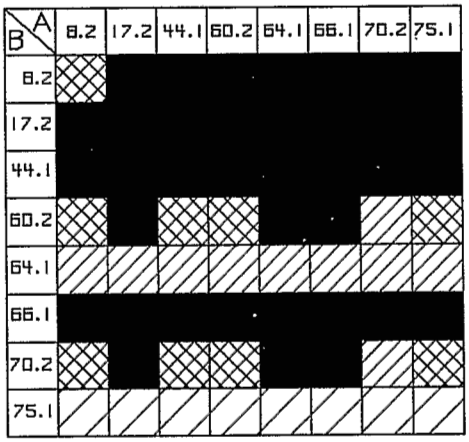
Nature of PCV epitopes recognized by mAb.

The reactivity of the mAb was analysed to establish whether the different mAb recognized PCV epitopes specific for intact virions (so-called neotopes (Van Regenmortel, 1982)), for depolymerized viral subunits (so-called cryptotopes) or for both forms of the protein (so-called metatopes). The reactivity

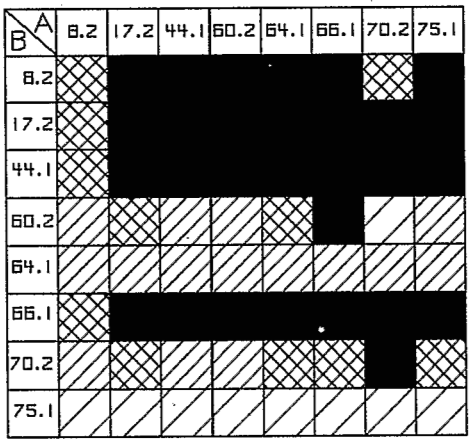
PCV K80



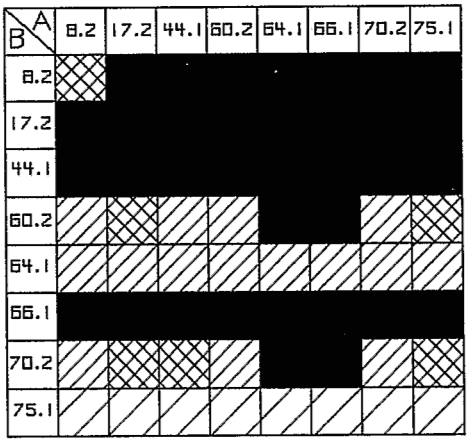
PCV K83



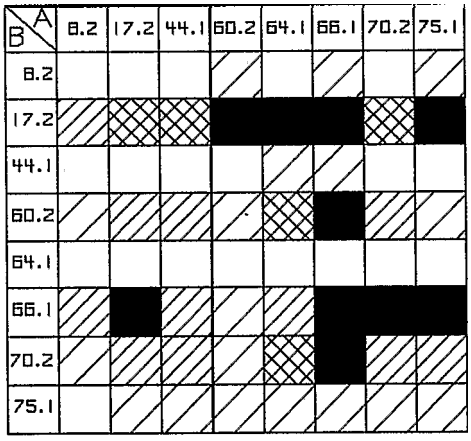
PCV MJ



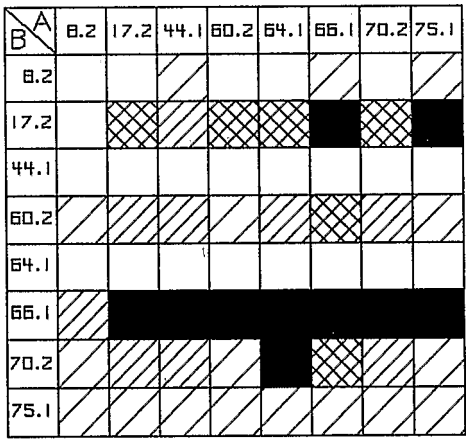
PCV 5



PCV N3



PCV N9



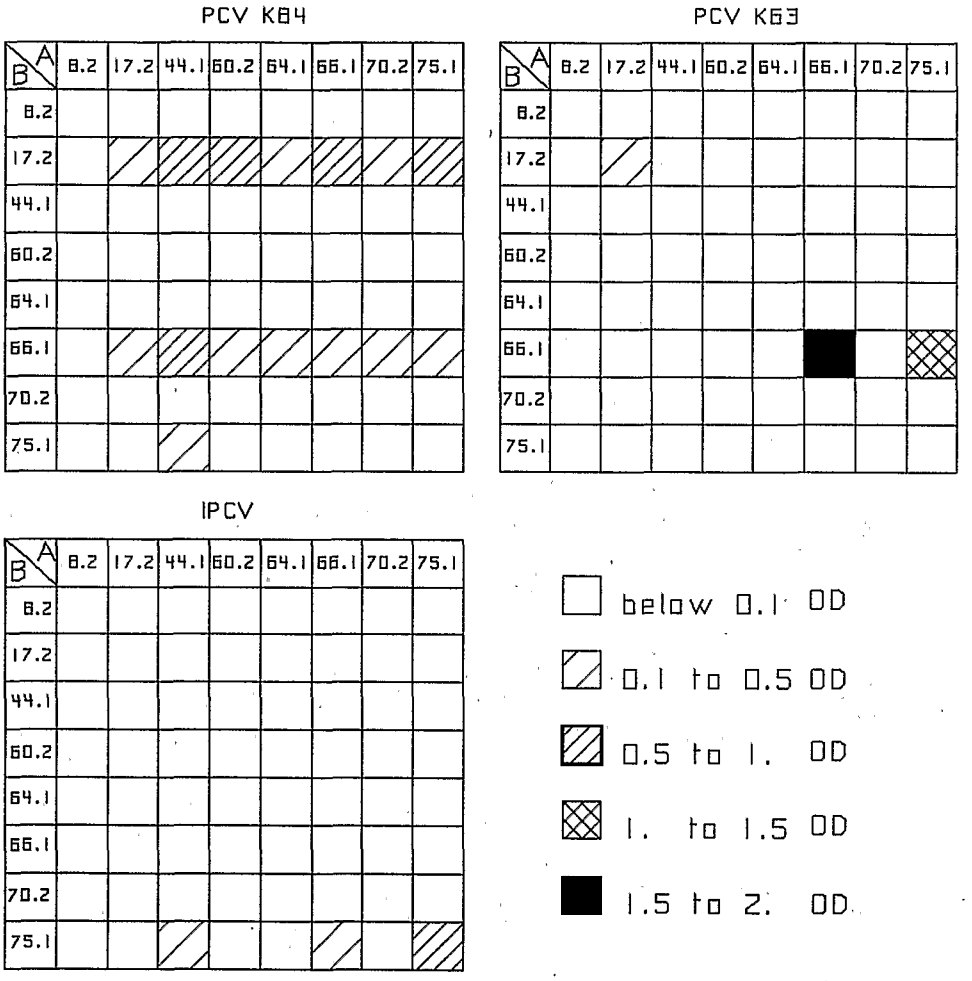


FIG. 4. — *Comparative reactivity of eight mAb tested against nine PCV isolates in ELISA procedure 6.*

Crude extracts of infected plants were used as antigen. The PCV isolates CHV and N2 did not react in this assay.

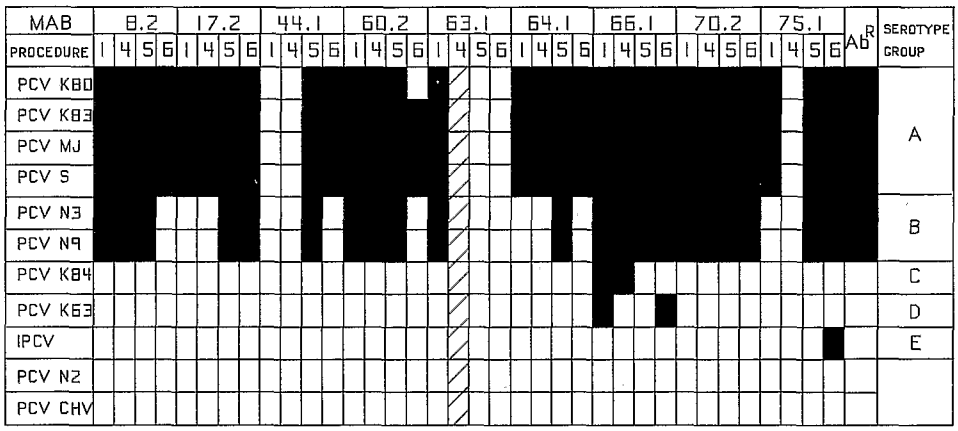


FIG. 5. — *Comparative reactivity of nine mAb tested against eleven PCV isolates in four ELISA procedures.*

Positive responses (■) correspond to OD values ⩾ 0.5; negative responses (□) correspond to values < 0.5. (▨): not tested.
Results shown for ELISA procedure 6 correspond to results obtained with the same mAb used as coating and labelled antibody. The column Ab^R correspond to DAS-ELISA with rabbit anti-K80 immunoglobulins.

of the 9 mAb characterized in figure 5 was tested by ELISA procedures 1 and 2 using intact virions and viral protein of isolate K80 as antigen. In procedure 1, the virus particles were considered intact, since they were kept at pH 7.0; in procedure 2, the virus particles were expected to be at least partly degraded by the alkaline pH of the coating buffer. In the case of tobacco mosaic virus, it has been shown by immunoelectron microscopy that partial degradation of the virus at pH 9.6 leads to preferential adsorption of protein subunits on the solid-phase in ELISA (Dore *et al.*, 1988). When PCV preparations were analysed by ELISA procedure 2, it was therefore expected that the antigen trapped on the microtitre plate was mainly dissociated viral protein.

The results shown in table II made it possible to distinguish six reactivity patterns in the mAb on the basis of their specificity for different forms of the viral antigen. Type I antibodies (mAb 64.1, 66.1 and 75.1) recognized the virions and the subunits in both ELISA procedures 1 and 2, and are thus specific for the type of epitope known as metatope (Van Regenmortel, 1982).

Type II antibody (mAb 17.2) also recognizes a metatope found on both virions and subunits in DAS-ELISA but no longer present on the protein subunits in ACP-ELISA. Presumably, the alkaline buffer (pH 11.0) used for preparing the viral protein together with the denaturation induced by adsorption of the protein to the microtitre plate (Dekker *et al.*, 1987; Dore *et al.*,

1988; Friguet *et al.*, 1984; McCullough *et al.*, 1985) destroyed the epitope conformation required for recognition by mAb 17.2.

Type III antibody (mAb 8.2) recognizes a type of metatope which is not destroyed by adsorption of the protein to the plastic in ACP-ELISA; however in DAS-ELISA, the viral subunit appears to be oriented by the trapping rabbit antibodies in such a way as to prevent mAb 8.2 from binding to its epitope. It should be noted that this type of steric hindrance was also observed when mAb 8.2 was used both as the coating and biotinylated antibody in DAS-ELISA (fig. 2).

Antibodies corresponding to types IV, V and VI recognize cryptotopes, *i.e.* epitopes found only on the viral protein. The cryptotope recognized by mAb 44.1 is present on subunits prepared at pH 11.0 as well as on subunits dissociated by the coating buffer in ACP-ELISA. In contrast, the cryptotope recognized by mAb 60.2 and 70.2 is present only on proteins treated at pH 11.0, whereas that recognized by mAb 63.1 is available only after adsorption of the protein to the plastic.

DISCUSSION

The mAb described in this report should be of considerable value both for the detection of PCV infections in the field and for discriminating between the different serotypes of this antigenically heterogeneous virus. The extreme antigenic heterogenicity of PCV is illustrated by the fact that two of the viral isolates included in the present study (N2 and CHV) did not react with any of the mAb nor with the rabbit antiserum raised against the K80 isolate. Both viruses were considered to be PCV isolates on the basis of symptomatology in groundnuts and *Chenopodium,* and electron microscope observations that indicated the presence of rod-shaped particles of the expected length (Thouvenel *et al.*, 1976). It is also noteworthy that the serotype distribution is not related to the geographic origin of the isolates; for instance, the four isolates K80, K83, K84 and 64 that were collected in the same field near Kirene in Senegal belong to three different serotypes. Since most of the mAb retained their reactivity when used for coating microtitre plates, a dual mAb-type assay would seem to be the most useful for the diagnosis of PCV-infected plants. For instance, if mAb 66.1k (ascitic fluid diluted 10^{-4}, see table II) is used for coating plates, it is possible to detect serotypes A, B, C, and D by means of biotinylated mAb 66.1 (diluted 10^{-5}) and serotype E by means of biotinylated mAb 75.1 (diluted 10^{-4}). This type of assay was found to be well suited for detecting virus in crude extracts of *Arachis hypogea* (groundnut), *N. benthamiana* and *C. amaranticolor.*

Various other combinations of dual mAb assays using the same mAb as coating and biotinylated antibody could also be used for detecting PCV serotypes A, B, D and E (see fig. 5). These findings corroborate data obtained with mAb to other plant viruses such as tomato mosaic virus (Dekker

et al., 1987), *Odontoglossum* ringspot virus (Dore *et al.*, 1987) and cucumber mosaic virus (Porta *et al.*, 1989) and clearly refute the claim made by Hill *et al.* (1984) and Diaco *et al.* (1986) that it is necessary in DAS-ELISA to use mAb specific for different epitopes of the virus as coating and labelled antibodies.

Our results also demonstrate the superiority of mAb compared to conventional antiserum (Van Regenmortel, 1986). As shown in figures 4 and 5, three additional PCV serotypes were detected when mAb 66.1 and 75.1 were used. Furthermore, when used in conjunction with ELISA procedures 1, 4 and 6, several of the mAb clearly differentiated between the different viral serotypes. However, it should be noted that the discrimination potential of the mAb was greatly reduced in ELISA procedure 5 (fig. 3) which gave results roughly equivalent to those obtained with rabbit antiserum.

The results summarized in figure 2 and table II illustrate the capacity of mAb to recognize minor changes in the antigenic structure of viral proteins. Although PCV is an extremely stable virus (Thouvenel *et al.*, 1976), the purification procedure used for obtaining virus particles clearly altered the epitopes recognized by mAb 8.2 and 75.1 (fig. 2). One consequence of this modification was that mAb 75.1 could not detect the virus in a purified preparation of PCV, although it readily detected it in a crude extract of infected *N. benthamiana*. This finding underlines the need to use the same type of antigen preparation in all serological comparisons between different viral isolates.

The ability of mAb to detect conformational changes in PCV protein brought about by changes in pH and by adsorption to a plastic surface is illustrated in table II. Such effects have been well documented (Al Moudallal *et al.*, 1984; Friguet *et al.*, 1984; McCullough *et al.*, 1985; Mierendorf and Dimond, 1983; Vaidya *et al.*, 1985) and again stress the importance of carefully selecting the ELISA format used for comparing the antigenic properties of different virus isolates.

RÉSUMÉ

ANTICORPS MONOCLONAUX SPÉCIFIQUES DE DIFFÉRENTS SÉROTYPES DU VIRUS DU «CLUMP» DE L'ARACHIDE

Des anticorps monoclonaux dirigés contre le virus du « clump » de l'arachide ont permis de caractériser 5 sérotypes du virus. La comparaison de 4 types de tests immunoenzymatiques ELISA a permis de sélectionner celui qui est le mieux adapté au diagnostic de routine et à la différenciation entre sérotypes du virus. La plupart des anticorps monoclonaux conservent leur activité après adsorption sur la phase solide en ELISA, et le même anticorps peut être utilisé comme capteur et comme anticorps biotinylé. Etant donné que les anticorps obtenus sont capables de reconnaître des changements mineurs de conformation de l'antigène viral, il est important de sélec-

tionner avec soin le format du test ELISA utilisé dans les comparaisons entre différents isolats du virus.

MOTS-CLÉS: Virus du «clump» de l'arachide, *Furovirus,* ELISA; Anticorps monoclonaux, Diagnostic, Phytopathologie.

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