The utilization of monoclonal antibodies in immunocapture RT-PCR and dot blotting immunobinding assays for the detection of *Cucumber mosaic virus*

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Haggag S. Zein*; M. H. Hussein*; H. A. Hussein* and Kazutaka Miyatake**

*Department of Genetics, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt. **Department of Applied Biological Chemistry. Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1, Gakuen-Cho, Sakai, Osaka, 599-8531, Japan. Corresponding author, email: haggagsalah@gmail.com

ABSTRACT

Reliable plant diagnostic techniques are necessary for good crop management. Occasionally, ELISA may not give a definitive result, requiring confirmation using different diagnostic methods. Immunocapture reverse transcription PCR (IC-RT-PCR) requires the direct confirmation of an ELISA result in a simple, cost-effective manner. We found that the use of captured antigens as a template in IC-RT-PCR can eliminate the need for traditional extraction methods and reduce the number of steps involved, costs incurred, and sampling variations. Experiments showed that a reliability of pathogens can be detected using ELISA in the IC-RT-PCR process. To date, a rapid assay for diagnosis of CMV has not been developed, and there is a critical need for a method that can be employed in both laboratory and field. Dot immunobinding assays (DIBA) are useful alternatives to microtitre plate enzyme-linked immunosorbent assay (ELISA). It has the additional advantages of simplicity, and is completed quickly in the field or the laboratory on large numbers of samples, in a short time. The method proposed provides a sensitive quantitative technique for dot-immunobinding assay.

Keywords: *CMV, Monoclonal Antibodies, Immunocapture-RT-PCR, Dot-immunobinding assay.*

INTRODUCTION

Plant viral diseases occur commonly in all crops. Some viral diseases have impacts on crop production by reducing yield or quality, while others have not notable effects. Plant viruses can cause serious losses to most, if not all, major crops. In addition, viral infections cause damage to fruits, leaves, seeds, flowers, stems and roots of many important crops. Most crop species are routinely infected with several different viruses (Brunt *et al.*, 1990). One of the most widespread viruses in horticultural crops is *Cucumber mosaic virus* (*CMV*). It is the type species of the *Cucumovirus* genus in the family of *Bromoviridae* (van Regenmortel *et al.*, 2000). *CMV* has a broad host range, a worldwide distribution and a severe impact on cultivated crops. These characteristics make it one of the economically important plant viruses in commercially grown crops (Palukaitis *et al.*, 1992). *CMV* expresses three genomic RNAs, designated RNA 1, RNA 2, and RNA 3, and at least two subgenomic RNAs, RNA 4 and RNA 4A, which are transcribed from the 3' portions of RNA 3 and RNA 2, respectively (Fig. 1).

Diagnostic techniques for viruses fall into two broad categories: biological properties related to the interaction of the virus with

its host and/or vector (e.g., symptomatology and transmission tests) and intrinsic properties of the virus itself (coat protein and nucleic acid). Detection methods based on coat protein include precipitation/agglutination tests; ELISA has been established well in our previous report (Zein *et al.,* 2006). Viral nucleic acid-based techniques like dot-blot hybridization assays and polymerase chain reaction are more sensitive than other methods. Availability of these diagnostic methods provides greater flexibility, increased sensitivity and specificity for rapid diagnosis of virus diseases in disease surveys.

Since PCR has the power to amplify the target nucleic acid present at an extremely low level and forms a complex mixture of heterologous sequences, it has become an attractive technique for the diagnosis of plant virus diseases (Henson and French, 1993; Hadidi *et al.,* 1995). The viruses detection with PCR differentiates at the family, genus, or strain level (Omunyin *et al.,* 1996), or for simultaneous detection of unrelated viruses in a sample by using a mixture of virus-specific primer pairs "Multiplex" PCR (Hauser *et al.,* 2000; Nassuth *et al.,* 2000). To limit nucleic acid loss, sample manipulation before PCR assays can be minimized by a direct release of viruses from minute amounts of excised tissues (Thomson and Dietzgen, 1995). Recently, many reports have shown that immobilized viruses (La Notte *et al.,* 1997; Olmos *et al.,* 1996; Rowhani *et al.,* 1995) or nucleic acid captured by magnetic beads (Regan and Margolin, 1997) increase the efficiency of RT-PCR and permit the use of large amounts of crude extracts.

Immunocapture (IC)-RT-PCR has been shown to be more sensitive than ELISA and direct RT-PCR for detecting viral RNA in infected plants (Wetzel *et al.,* 1992).

Dot immunoblotting assay (DIBA) can be used to detect viruses in both plants and vectors (Makkouk *et al.,* 1993). The technique is similar to ELISA except that the plant extracts are spotted onto a membrane rather than using a microtitre plate as the solid support matrix. Unlike in ELISA, where a soluble substrate is used for color developpment, a precipitating (chromogenic) substrate is used for virus detection in the DIBA. Hydrolysis of chromogenic substrates results in a visible colored precipitate at the reaction site on the membrane. Chemiluminescent substrates, which emit light upon hydrolysis, can also be used and the light signal is detected with X-ray film as with radio labelled probes (Leong *et al.,* 1986). An optimized DIBA is as sensitive as ELISA, simple, relatively inexpensive and the results can be scored visually.

The purpose of this research was to study the sensitivity of *CMV* diagnosis with dot-immunoblotting assay and immunocapture RT-PCR, in particular with the production a monoclonal antibody and to determine the sensitivity and specificity of the dot-immunobinding assay, to compare its validity, and quantify *CMV* in infected plant sap. Knowledge of the specific immuno-globulin genes for common epitope may lead to insights on pathogen-host co-evolution and helps block the virus infections in plants.

MATERIALS AND METHODS

Virus purification and storage

Tobacco plants (*N.tabacum* cv. Xanthinc) and *N. benthamiana* plants at the five-leaf stage were used for inoculation. The pepo strain of *CMV* (subgroup IA) was originally obtained from *C. pepo* in Japan (Saiga *et al.,* 1998); *CMV* propagated in tobacco was purified as described by (Takanami, 1998). Plants were inoculated mechanically with either purified *CMV* strain pepo or m2, diluted to a final concentration of 50 μg ml^{-1} in 100 mM phosphate buffer, pH 7.0. Inoculated

plants were grown in a growth chamber (NK systems) at 24°C with a 14 hr light/10 hr dark cycle. Virus accumulation was assessed at 5 days post-inoculation for strain pepo and one month post-inoculation for strain m2.

Monoclonal, polyclonal antibodies production

Hybridomas producing antigen-specific antibodies were screened by ACP-ELISA. The screening tests were performed four times during cell growth in HAT selection medium (Gibco). Positive clones were subcloned twice by the limiting dilution method. Cells positive were transferred to a 24 well plate and further sub-cultured in HT medium (Gibco). Supernatants were screened using ELISA plate coated with *CMV* at a concentration of 1 μg/ml. Wells with single colonies were microscopically selected and the antibody secretion was confirmed by *CMV* binding assays in ELISA and by western blotting. Subsequently, stocks were made for high secretors and used for further steps. One hundred thirty of 1340 wells from five fusions showed recognition to *CMV* coat protein, and 14 wells among these 130 wells showed inhibition of antibody binding by either of the two *CMV* sub-groups.

Purification of mouse monoclonal antibodies (mAbs)

A method suitable for the isolation of monoclonal antibodies from large volumes of serum-containing hybridoma cell culture supernatants was carried out in three steps: precipitation with saturated ammonium sulfates, HiTrap affinity chromatograph, and gel filtration on Sephacryl S-200 HR. Bulk production of monoclonal antibody was obtained by giving an intraperitoneally injection of ~10^7 hybridoma cells into the cavities of eight weeks old BALB/c mice primed 2 to 3 weeks previously with 0.5 ml pristine (2, 6, 10, 14-tetramethylpentadecane) and the antibodies were isolated from the isolated ascitic fluid accordingly as previously described (Harlow and Lane, 1988). While the purifications of IgM were performed from the culture supernatants, and were treated with saturated 45% ammonium sulfate for precipitated IgM, suspended in 0.15 M borate buffer and applied to a gel filtration column (Sephacryl S-300), with a flow rate of 0.25 ml/min. The fractions containing IgM were collected and the absorbance was monitored at 280 nm. The extinction coefficients for 1 mg/ml solution were 1.4 for IgG and 1.2 for IgM.

RT-PCR

Analysis was done according to Jansen *et al.* (1990). First strand cDNA synthesis was carried out by adding 0.5 U of M-MLV reverse transcriptase and 2 U of RNase inhibitor (TOYOBO, Osaka, Japan) with primer P3R123Bam (5′-CCGGATCCTGGTC-TCCTTTTGGAGGCCC-3′) which is complementary to 3′ proximal 20 nucleotides of *CMV* RNAs according to Nitta *et al.* (1988), and incubation at 37°C for 90 min was performed. Nested PCR reaction was done using an oligonucleotide specific to genomic RNA3 pepoCPF (5′-CCTCTAGACTTCTCCGCGA-GATTGCG-3′) and p3CPR3/4 (5′-CCGA-GCTCTCAAGCTTGAACTCCAGATGCAG-3′). The amplification product was completed up to 100 μl with distilled water. The PCR reaction mixture was prepared by adding 1.2 μl (2.5 mM dNTPs mix); 1.2 μl $MgCl_2$ (25 mM); 1.2 μl each primer (10 mM) as mentioned above;1 μl of the cDNA; 1.2 μl high buffer (10×); 0.2 μl Taq-DNA polymerase 5 U/μl; and nuclease free distilled water up to a final volume 12 μl. The cycling program was 5 min at 94°C without added Taq-polymerase and subsequently, 30 cycles after adding Taq-polymerase (92°C, 58°C, and

72°C for 30 sec, respectively) and then final incubation for 5 min at 72°C. Nested PCR amplification products were viewed under UV light after electrophoresis in 1% agarose gel Tris-Acetate-EDTA buffer and stained with ethidium bromide. All other chemicals were purchased from (Nacalai Tesque, Inc. Kyoto, Japan).

Dot-immunobinding assay

Young upper leaves of infected and non-infected plants were homogenized with extraction buffer and purified *CMV* diluted with healthy plant sap 10^{-2} were gently dropped (2 μl, each) onto membranes of PVDF of 0.45-μm pore size (Bio-Rad). The membrane was air-dried and blocked for 30 min in TBS buffer (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl) containing 50 g L^{-1} defatted milk powder (TBS-milk buffer). Membranes were incubated for 60 min in 1μg/ml mAbs diluted with TBS-milk buffer. After three washes of 5 min each with TBST (TBS plus 3 g L^{-1} Tween-20) and two washes; the first and the second with distilled water, membranes were incubated for 60 min in a 1/5000 dilution in TBS-milk buffer with appropriate Goat anti-mouse alkaline phosphates. AP-conjugated antibody (Amersham, Pharmacia, Biotech). Membranes were then washed as before and equilibrated in substrate buffer (0.1 M Tris-HCl, pH 9.5) for 5 min before adding the substrate. All incubations were at room temperature. AP activity was detected with the chromogenic substrate (5-bromo-4-chloro-3-indolyl phosphate/4-nitro-blue tetrazolium chloride BCIP/NBT.

RESULTS AND DISCUSSION

Antibody production, purification and characterisation

The antibodies bind a broad variety of antigens with high affinity and specificity and the structural information about the molecular interactions between the antibodies and antigens helps to understand the effect of mutations on the affinity and specificity of the antibody. Monoclonal antibodies (mAbs) were prepared against *CMV* coat protein Japanese m2 and pepo strains (Table 1). In addition, mAbs were characterized and used to develop a diagnostic high sensitivity and specificity against virus coat protein with ELISA (Zein *et al.*, 2006). Fusion of splenocytes of immune mice with X63Ag8.653 myeloma resulted in a number of hybridomas, allowing the production of hybrid cell lines from B cells which secret a single monoclonal antibody with one binding site specifically, further, can potentially produce unlimited quantities. However, the serological differentiation of *CMV* isolates is of importance in breeding for disease resistance and in studying disease epidemiology, therefore specific monoclonal antibodies have been developed, one of the goals, in their development was to provide tools for serotyping isolates of the virus (Table 1). Ten clones were selected for the study because their anti-*CMV* activities were detected by solid phase ELISA and western blotting, when the culture supernatants were diluted 100-1000-fold. Antibodies of these clones were purified from ascitic fluid by affinity chromatography (Figs. 2 and 3). The data in (Table 1) indicate that the selected antibodies bind to three different epitopes of *CMV*.

Where, mAbs- (4, 5, 6, 7, 8, and 10) were bound specifically to pepo-*CMV* (same epitope or adjacent epitopes); no cross reactivity with other strains were detected. Further, mAbs- M2- (1, 2, and 3) were bound specifically to m2-*CMV*. On the contrary, M2-4 antibody showed cross reactivity with both strains m2- and pepo- *CMV* consequently. This mAbs showed high reliability to detect various strains and isolates of native *CMV* particles using an immunobinding-dot binding.

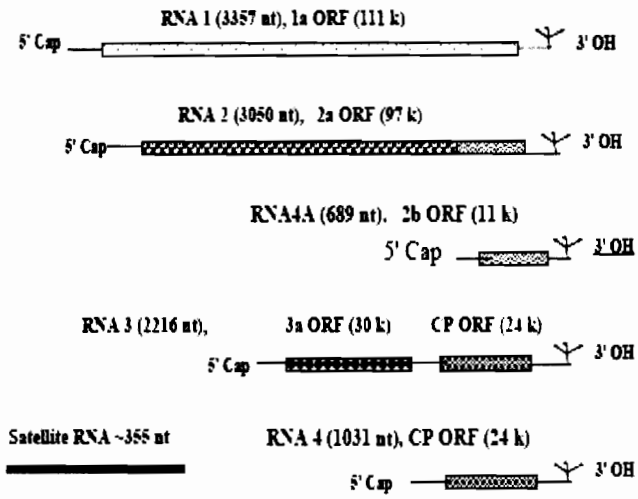

Fig.(1): Genome organization of CMV. The nucleotide and amino acid numbers are for Fny strain; RNA 1,2, and 3 are genomic and required for infection, RNA4 and 4A are subgenomic RNA4 packaged in virions of all strains, RNA4A only packaged in subgroup II strains (Roossinck, 2002).

CMV detection with IC-RT-PCR

In IC-RT-PCR, the Immunocapture of virions from crude plant extracts was carried out directly in PCR tubes in a fashion reminiscent of the ELISA assay. It is a simple and sensitive analysis of multiple samples without a preliminary purification of the virus particles or viral RNA. This step has concentrated and pre-purified the virus particles. RT-PCR was then undertaken on the trapped virus particles omitting the need for nucleic acid extraction. When compared with ELISA, IC-RT-PCR improved detection sensitivity by several orders of magnitude (Wetzel *et al.*, 1992; Jacobi *et al.*, 1998). Based on these promising results, we decided to develop a sensitive method of IC-RT-PCR test for *CMV* detection in infected tobacco plants. It was hypothesized that IC-RT-PCR would improve both detection sensitivity and detection specificity for *CMV*, when compared with serological methods. The IC-RT-PCR procedure was first optimized as to the dilution of coating antibodies, plant saps, concentration of reverse transcriptase, number of amplification cycles and annealing temperature. A dilution of mAb-5 at 1:200 and plant saps at 1:10 revealed that the optimum condition for detection of *CMV* with IC-RT-PCR, using primer pair pepoCFPF/p3CPR; the investigation of the effects of temperature, results indicated that the critical point in this experiment is the temperature for the virus particle lyses. A range of temperatures from 65°C to 90°C with different time exposures from 5 min up to 30 min, 65°C for 25 min gave the optimal detection and it was possible to specifically detect purified viruses as well as virus infected tobacco plants (Fig. 4). In both cases, the products observed were of the correct predicted size of about 595 bp. The relative sensitivities of IC-RT-PCR were compared by using serial dilutions of purified pepo-*CMV* (100 ng/ml), followed with serially ten-fold diluted lanes 1 to 5 respectively; with a minimum detection limit of 0.01 ng/ml (lane-5). No reaction occurred when the virus was substituted with buffer lane-6 (Fig. 4-A). On the other hand, for the detection of CMV infected plant sap, we investigated the crude extract RNA concentrations on specific amplification by testing different dilutions of the standard crude extract. The dilution rate of infected plants: 1/100 correctly predicted a size of about 595 bp, as shown in (Fig. 4-B).

Table (1): Cucumber mosaic virus immunoglobulin classes and subclasses of onoclonal antibodies, and the cross reactivity to CMV subgroups.

Fusion	Clones	Immunogen	Subclass	Optimum	Subgroup*	
				µg/ml	I	II
1	4	Pepo	IgG1	0.20	++	–
	5			0.05	+++	–
	6			0.15	+++	–
2	8	Pepo	IgG1	0.45	++	–
3	7	Pepo	IgG2b	0.80	+	–
4	M2-1	M2	IgG1	2.0	–	++
	M2-2			2.0	–	++
5	M2-3	M2 +	IgG1	5.0	–	+
	M2-4	Pepo	IgG1	10.0	++	++

* The specific reactivity of the MAbs specific binding activity against *CMV*-CP. The prescreening of the MAbs with ELISA were scored as (+ or -) for measurements corresponding to absorbance (450 nm) values < 0.5 (-), 0.5-1 (+), 1-1.5 (++), 1.5 -2 (+++).

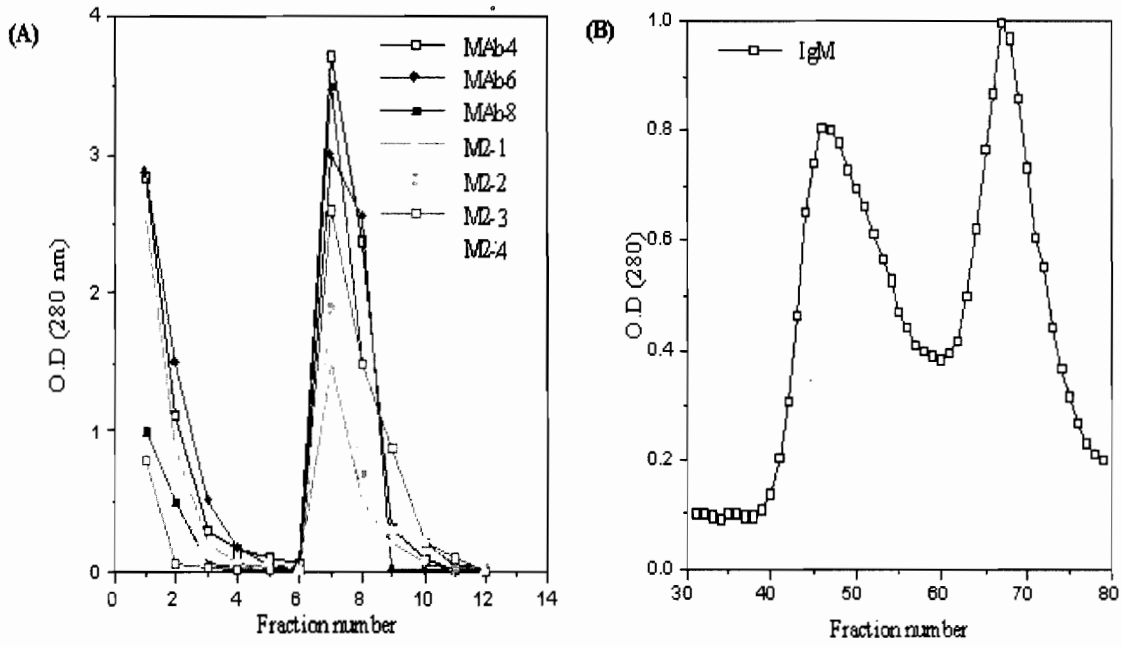

Fig. (2): The purification of monoclonal antibodies IgG and IgM (A) MAbs production with ascitic fluids and purified using HiTrap affinity protein A, (B) Culture supernatant of serum free media for producing IgM and purified with gel filtration on Sephacryl S-300, IgM fractions monitored with absorbance O.D $_{280}$.

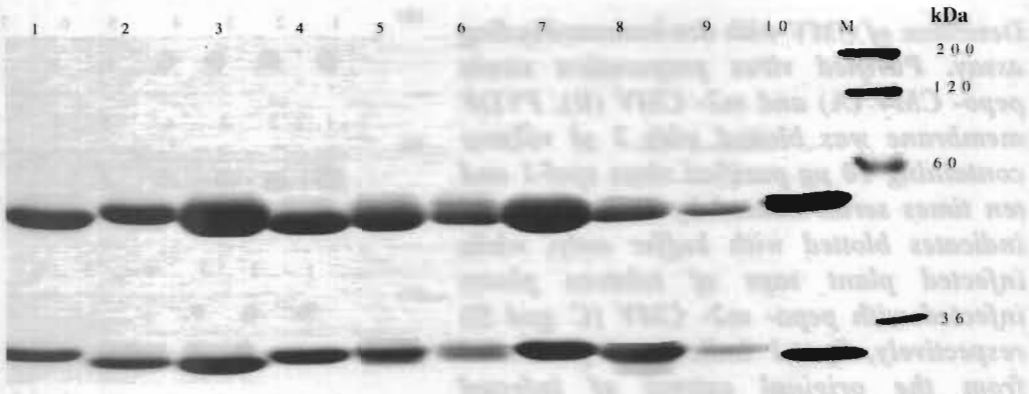

Fig. (3): The purification of the monoclonal antibodies specific CMV coat protein with affinity protein-A column. Purified mouse monoclonal immunoglobulins IgG were separated on 10% SDS-PAGE under reducing condition (2- mercaptoethanol) followed by staining with Coomassie Brilliant Blue R 250. MAbs- (4, 5, 6, 7, and 8) numbered 1 to 5, while MAbs-M2 (1, 2, 3, and 4) numbered 6 to 9, while PAbs-IgG numbered 10. Molecular marker in kDa is indicated on the right side.

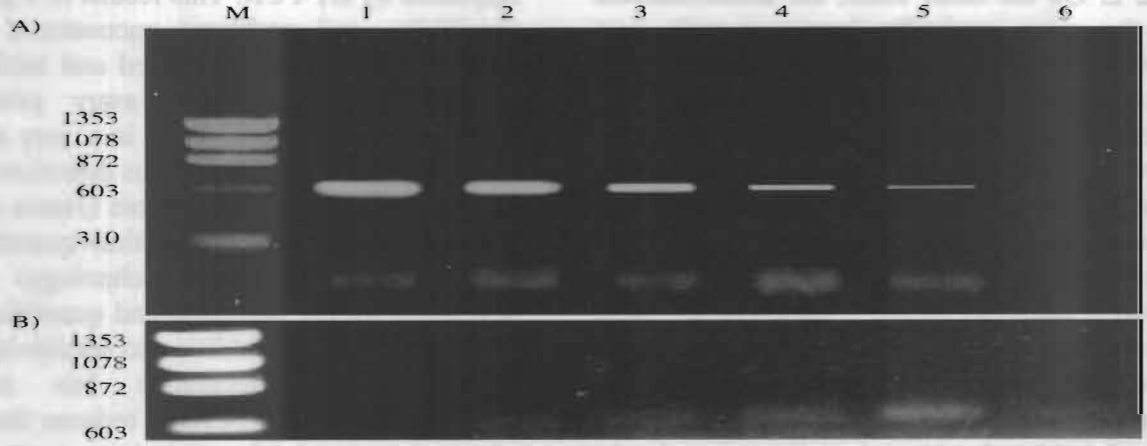

Fig. (4): Sensitivity and specificity of IC-RT-PCR for detection of CMV. All tubes were coated with purified mAb-5 and all RT-PCR reactions contained primer pairs with reaction mixture. (A) The final concentrations of 100-, 10-, 1-, 0.1-, and 0.01- ng/ml for lanes 1 to 5 respectively, and the reaction was substituted, the RNA with buffer in lane 6. (B) Detection of CMV in inoculated tobacco plants (dilution rate: 1/100). Healthy plant sap was in lane 1, the time post inoculation 12; 24; 48; and 96 hr, were lanes 2 to 5 respectively, infected tobacco plants sap diluted at 10^{-5} was in lane 6. M; Molecular weight φx174 digested with Hae III, with their size in base pairs on the left side.

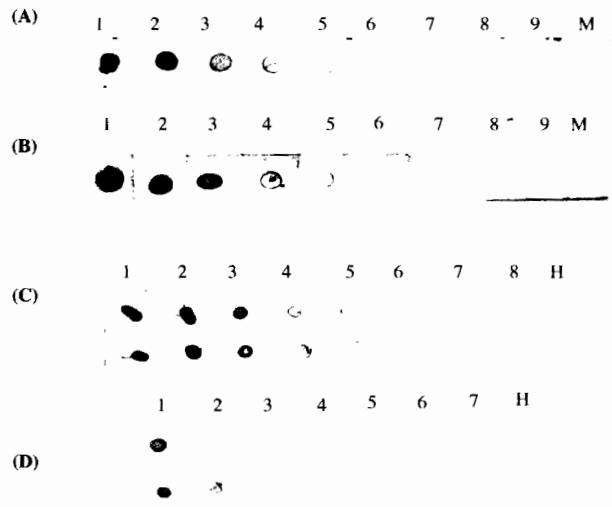

Fig. (5): Detection of CMV with dot-immunobinding assay. Purified virus preparation strain pepo- CMV (A) and m2- CMV (B). PVDF membrane was blotted with 2 µl volume containing 10 µg purified virus spot-1 and ten times serial diluted by TBS buffer. M indicates blotted with buffer only, while infected plant saps of tobacco plants infected with pepo- m2- CMV (C and D) respectively, Spot-1 indicates 3 µl blotted from the original extract of infected tobacco plant sap diluted 1/10 pepo- CMV (C) or m2- CMV (D) and followed with ten time diluted each spot. H indicates healthy tobacco plant sap.

In addition, importantly, twelve hours post-inoculation treatment was obviously enough to detect the virus by this method for lane 2. On the other hand, the accumulations of the virus gradually increased with post inoculation time (lanes 3 to 5, respectively). Remarkably, a high dilution rate 10^{-5} of infected plant sap was detected in lane 6; however, there was no detection in the healthy plants (lane-1). The IC-RT-PCR approach was chosen rather than hybridization or RT-PCR tests, because of high sensitivity due to an initial immunocapture enrichment step followed by PCR amplification. The high specificity was due to the combination of virus specific antibody captures and primer specificity, and virus detection directly in crude plant extracts rather than in partially or highly purified nucleic acid preparations. Efficacies of these treatments appear to vary substantially between different viruses, and therefore, the best conditions for any particular virus are difficult to predict and need to be assessed individually. Effective control of viral infection in plants depends on selecting and propagating virus-free plants and eradicating diseased plants. In this assay, the virus particles are first "concentrated" by trapping onto a solid surface (either microcentrifuge tube or ELISA plate) using virus specific antibodies. The trapped virus particles are disrupted and the released viral nucleic acid is amplified by RT-PCR. This results in a greater sensitivity, and problems encountered with RNA extraction are minimized and inhibitors of RT-PCR are washed away prior to amplification. Thus, IC-PCR is a very useful alternative for RT-PCR in virus detection from plant material and insect vectors (James *et al.*, 1997). Recently, a novel real-time quantitative PCR assay (TaqMan technology) was developed for the detection and quantification of plant viruses. In addition to sensitivity and specificity, this technique has certain advantages over RT-PCR; it reduces the risk of cross-contamination, obviates post PCR manipulations, provides higher throughput, and enables quantification of virus load in a given sample. However, this technology requires expensive and special equipment and reagents compared with conventional PCR technology.

Detection of CMV with DIBA

The sensitivities of mAbs were compared for detection of *CMV* which could be readily detected both in purified virus preparations and in crude sap of infected tobacco leaves by

DIBA on PVDF membrane (Fig. 5). The serial dilution of the purified *CMV* 10 μg ~1 pg, the detection levels were 1 pg (1 ng/ml) with purified preparation of pepo-*CMV* (Fig. 5-A), while infected leave extracts were detected at 10^{-5} dilution of the same strain (Fig. 5-C). The detection of m2- *CMV*, when tested with DIBA as well, was shown to be lower than pepo-*CMV*. The minimum detection levels were 1 ng (1 μg/ml) (Fig. 5-B), but, the infected leaves revealed a detection limit at 10^{-3} dilution (Fig. 5-D). The sensitivity of the different antibodies was compared for detecting the virus in infected tobacco plants. The results were obtained by limiting dilution of the infected plants with pepo- *CMV*. mAbs-(4 and 5) showed the highest specific reactivity limited detection at 10^{-4} dilution, mAbs- (6, 7, and 8) showed intermediate limit detection at10^{-3} dilution while mAb-10 revealed the lowest detection limit at 10^{-2} dilution (Data not shown). *CMV* -m2 specific mAbs showed a lower delectability by limiting the dilution range 10^{-1}~10^{-3} (Fig. 4-B). Dot-immuno-binding assays have been shown to be advantageous because they are simple, sensitive, and rapid. They are widely used in the field of clinical chemistry and cell biological studies. The protein of interest is detected by reaction with a primary antibody .followed by a labeled second antibody. The second antibody is conventionally labeled by enzyme; consequently, it is a potentially useful research and diagnostic tool in plant pathology, particularly in the study of pathogens transmitted by vectors. The low volume of the sample required for dot-immunobinding assays is an important advantage over ELISA in analyzing samples without dilution. Moreover, the dot-immuno-binding assay is a simple immune-assay method to detect and quantify *CMV* coat protein in infected plants. The immune binding activity of a panel of mAbs raised to *CMV* has been examined in ELISA (Zein *et al.*, 2006). The sensitivity of the immune-assays was compared with that using infected plant extracts or purified virus preparations, and as little as 500 pg coat protein could be detected. One advantage of the immune-dot blot technique is that the membranes used to bind the proteins also allow their retention of antigenicity and accessibility to antibody. Direct coating of polystyrene or polyvinyl chloride ELISA plates with the protein of interest can result in the deformation of the antigenic epitopes against which the mAbs react to avoid this potential problem. A sandwich ELISA is often designed, so that the microtiter plates are required to be coated with capture antibodies that serve to anchor the protein of interest and the antigen retains its native conformation.

Another improvement of the DIBA described here over the sandwich ELISA is that fewer antibody reagents are necessary for performance of the test; nitrocellulose can bind greater quantities of protein per surface area than polystyrene or polyvinyl chloride plates, and it binds protein quantitatively up to concentrations of 80 mg/cm^3. Attempts to improve the sensit-ivities of immunoassays usually focus on optimization of detection by varying the levels of antibody, by using antibody that is more specific to the antigen, and by using different enzyme conjugate-substrate systems including signal amplify-ication schemes. Of the techniques assessed for their sensitivity and applicability for the detection of *CMV* in infected plant tissues, they were protein-based and relied entirely on the use of polyclonal antiserum or monoclonal antibodies.

Consequently, nucleic acid-based diagn-ostic assays may be the methods of choice. Virus detection and identification are of prime concern for disease control. Simple immunolo-gical methods, such as ELISA and DIBA are

popular for virus detection. However, antiserum specific to each virus is required for success with these methods. Furthermore, they must be done indepen-dently for, species if multiple virus detections are required. The polymerase chain reaction method is a sensitive and rapid method for virus detection. In the case of *Tomato spotted wilt virus* detection, reverse transcription-PCR (RT-PCR) can detect lower levels of virus concentration than ELISA. Furthermore, two or more targets can be discriminated using a multiplex RT-PCR, a method that simultan-eously amplifies cDNA fragments of multiple sizes. Several previous reports have addressed the use of multiplex RT-PCR; for multiple virus detection in a single reaction (Wetzel *et al.*, 1992; Jacobi *et al.*, 1998).

CONCLUSIONS

Nine monoclonal antibodies were obtained from five fusion experiments performed after immunizing mice with two Japanese virus strains, pepo-, m2- *CMV* as antigens. These mAbs showed a diverse reactivity for the different subgroups of the isolates. Of these mAbs, five mAbs specifically reacted with *CMV* subgroup-I, three mAbs were capable of identifying the subgroup-II. One of the monoclonal antibodies was found to be non-specific for *CMV* -coat protein. We compared them to detect the limit of the pure virus protein, DIBA and IC-RT-PCR which was the most sensitive that detected a 10 pg/ml for the pure virus protein, while for other methods, levels were 10 ng/ml. From the results, we concluded that the present methods are very useful for diagnosis of infected plants.

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الملخص العربي

استخدام تفاعل البلمرة المتسلسل المعكوس المقترن باستخدام الأجسام المضادة لتشخيص فيروس تبرقش أوراق الخيار

*حجاج صلاح زين مصطفى ، *هاشم احمد حسين، *منى هاشم حسين و **كاظوتاكا مياتاكى
*قسم الوراثة – كلية الزراعة – جامعة القاهرة
**قسم تطبيقات التكنولوجيا الحيوية – كلية الزراعة وعلوم الحياة – جامعة اقليم أوساكا – اليابان

تقنيات تشخيص الأمراض الموثوق منها فى النبات ضرورية لتحسين المحاصيل. فمثلاً ELISA لا تعطى نتائج دقيقة وتحتاج إلى زيادة تأكيد باستخدام طرق تشخيصية أخرى . فمثلاً (IC-RT-PCR) تسمح بتأكيد نتائج الـ ELISA مباشرة بطريقة سهلة. فوجدنا أنه باستخدام الـ captured antigens يمكن أن تستخدم لمجسات فى تفاعل IC-RT-PCR وهذا يؤدى إلى الإستغناء عن طرق الإستخلاص الفيروسات التقليدية ، وأيضا يقلل كل من عدد الخطوات اللازمة لذلك ، والتكلفة وتباين العينات وبذلك فان مقدرتنا على إستخدام نتائج DAS-ELISA فى IC-RT-PCR يمدنا بطريقة بديلة لإستخلاص الفيروس عندما لا يوجد نباتات إضافية أو نباتات وفيرة لكى نقوم بالاختبار التأكيدى . وهذا يمدنا بمرونة أكثر لعمل الاختبارات التأكيدية . ونتائج هذه التجارب المعروضة في هذا البحث تعرض لنا ذلك أيضا وتأكد أن وجود الكائنات الممرضة ممكن أن يلاحظ باستخدام ELISA فى IC-RT-PCR . وحتى يومنا هذا لا يوجد طريقة سريعة لتشخيص الـ CMV فهناك ضرورة قصوى لتشخيص هذا المرض فى كل من المعمل والحقل . بينما طريقة الـ DIBA طريقة بديلة للـ ELISA ولها ميزة إضافية وهى السهوله بالاضافة إلى أن تفاعلها يكتمل بسرعة فى كل من الحقل والمعمل وبكمية كبيرة من العينات وكل هذا فى فترة وجيزة جدا. و تمدنا الطريقة المعروضة بتقنية تشخيص كمية وحساسة لـ Dot-immuno binding assay.