

COMPARISON OF ELISA AND RT-PCR ASSAYS FOR DETECTION AND IDENTIFICATION OF CUCUMBER MOSAIC VIRUS (CMV) ISOLATES INFECTING HORTICULTURAL CROPS IN POLAND

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A B S T R A C T

The suitability of DAS-ELISA and RT-PCR methods for detection of 15 cucumber mosaic virus (CMV) isolates found in Poland was compared. The effectiveness of DAS-ELISA depended on polyclonal antibodies (PAb) used in the assay. Antibodies Wic and DTL detected all tested isolates, whereas antibodies M could not detect isolate P26, antibodies ToRS did not react with isolate Porz, and isolate Simp2 was not detected by antibodies Cas. The RT-PCR technique allowed detection of all virus isolates. Three nucleic acid extraction methods: immunocapture (IC), silicacapture (SC) and isolation of the total RNA with the commercial kit (RN), proved to be effective. These three nucleic acid extraction methods all allowed CMV amplification by RT-PCR. Three methods were used to identify and group the CMV isolates: 1) ELISA with group-specific monoclonal antibodies (MAbs), 2) phylogenetic analysis of coat protein gene sequence and 3) restriction analysis of PCR-amplified RNA2 and RNA3 fragments digested with *EcoRI*, *HpaII* and *MluI* enzymes. MAbs used in ELISA allowed the preliminary classification of isolates to group I or II, but they failed to recognize one isolate (P26). CMV grouping based on CP sequence analysis enabled us to identify all of the tested isolates and discriminate between the two groups of CMV – IA and II. Classification based on RT-PCR-RFLP analysis correlated with phylogenetic grouping based on sequencing.

Key words: cucumber mosaic virus, DAS-ELISA, immunocapture, silicacapture, RT-PCR, RFLP, sequence analysis

INTRODUCTION

Cucumber mosaic virus (CMV), a member of the *Bromoviridae* family, is distributed throughout the world. It has the largest host range of all plant viruses, causing many diseases in vegetable, fruit and ornamental crops, with severe economic losses. CMV is readily transmitted in a non-persistent manner by more than 75 species of aphids (Palukaitis et al., 1992). Various isolates, differing in host range and pathogenicity, have been described. They fit into three major groups: IA, IB and II (Palukaitis and Zaitlin, 1999). Generally, groups IA and IB are predominant in the tropics and subtropics and they cause severe symptoms and epidemics (Hu et al., 1995; Singh et al., 1995; Varveri and Boutsika, 1999). Group II isolates are prevalent in temperate regions (Haase et al., 1989). Several methods have been used to detect CMV and differentiate virus groups: serology (Tóbiás et al., 1982; Haase et al., 1989; Porta et al., 1989; Wahyuni et al., 1992) nucleic acids hybridization (Piazzola et al., 1979; Owen and Palukaitis, 1988), peptide mapping of the coat protein (Edwards and Gonсалves, 1983) and lately RT-PCR (Wylie et al., 1993) combined with the RFLP (Finetti Sialer et al., 1999; Bashir et al., 2006) or sequence analyses (Roossinck, 2002; Srivastava and Raj, 2004; Bashir et al., 2006).

In Poland diseases caused by CMV have been known about since 1957 (Kochman and Stachyra, 1957).

The virus was detected in several vegetable crops, including cucumber, pepper, lettuce, spinach (Twardowicz-Jakusz, 1971; Twardowicz-Jakusz et al., 1986; 1996; 2003), in lupin (Książek, 1963) and red currant (Śliwa et al., 2008), as well as several ornamental plant species e.g. gladiolus, lily, impatiens, begonia and honeysuckle (Kamińska, 1976; 1995; 1996; Kamińska et al., 2005; Korbin and Kamińska, 1998). The virus was also found in several annual and perennial weeds which were identified as potential secondary reservoirs of viral infection (Twardowicz-Jakusz, 1971; Błaszczak and Mańka, 1977). Until recently CMV isolates were detected using biological tests (Twardowicz-Jakusz, 1971; Kamińska, 1976) or serological methods, mainly ELISA (Kamińska, 1995; Korbin and Kamińska, 1998). There is little information on the effectiveness of immunoassay using different antibodies for detection of a wide range of CMV isolates occurring in Poland. The suitability of virus detection systems based on analysis of nucleic acids has not been evaluated so far. Molecular techniques were used for identification of only a few CMV isolates (Borodynko et al., 2004; Pospieszny et al., 2004; Śliwa et al., 2008).

In this study we compared and evaluated ELISA with several polyclonal and monoclonal antibodies and three variants of RT-PCR (IC, SC and RN) for sensitive detection and grouping of CMV isolates.

MATERIAL AND METHODS

CMV isolates

Cucumber mosaic virus isolates originated from the collection of viruses at the Research Institute of Pomology and Floriculture, Skierniewice, Poland. They were originally found in different plant species: lily (isolate Cas, CB, P26, Simp2), cucumber (J, M), honeysuckle (Wic, WicDS), impatiens (Imp), dahlia (D), red currant (Porz) and tulip (Inz). CMV isolates from pumpkin (Dn) and cucumber (Og) plants (kindly provided by Prof. H. Pospieszny, Institute of Plant Protection, Poznań, Poland), as well as the isolate Del originating from the delphinium plant (obtained from Prof. J. Stanulis, Institute of Botany, Vilnius, Lithuania) were included in this study. All isolates were inoculated into *Nicotiana rustica* L. plants and maintained in a greenhouse during the experimental period. Isolates of *Peanut stunt virus* (PSV), *Tomato aspermy virus* (TAV) and healthy tobacco plants were used as the controls.

ELISA

Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) protocol of Clark and Adams (1977) was applied in all the experiments. Polyclonal antibodies (PAb) used for detection of the tested isolates were:

- DTL and ToRS, Loewe Biochemica, Germany;
- Cas, Korbin and Kamińska, 1998 and;

- M and Wic, Berniak et al., in press.

For isolate identification two sets of monoclonal antibodies (MAb) (Agdia, Biokom, Poland) were applied: 1) CMV-I allowing identification of isolates from group I (formerly described as the DLT serogroup) and 2) CMV-II – for detection of group II (serogroup ToRS) isolates.

Template preparation, reverse transcription and PCR amplification

Three procedures of nucleic acid preparation for RT-PCR were used:

- IC – the immunocapture protocol of Candresse et al. (1995) with modifications by Malinowski (2005), using a mixture of CMV-specific IgGs M and Wic as coating antibodies;
- SC – the silicacapture method described by Boom et al. (1990) with adaptations by Malinowski (1996);
- RN – total RNA extraction using the RNeasy Plant Mini Kit (Qiagen, Syngen, Poland).

Reverse transcription and PCR amplification were performed using Titan One Tube RT-PCR System (Roche Diagnostics, Poland). The parameters of RT-PCR and preparation of working solutions were done according to the manufacturers' instructions. Reactions were carried out with *Cucumovirus*-specific primers pair CPTALL5-CPTALL3 (Choi et al., 1999) as well as five pairs of CMV-specific primers: CMV1-CMV2 (Wylie et al., 1993), D3F-D3R (Graves and Roossinck, 1995), RW8-RV11

(Finetti Sialer et al., 1999), M1-M2 (Lin et al., 2004) and 3a780F-CP50R (Śliwa et al., 2008). Primers CPTALL5-CPTALL3 and CMV1-CMV2 allowed amplification of RNA3 fragments containing the coat protein gene (CP), primers M1-M2 and D3f-D3R – fragments of the movement protein (3a) gene, primers RW8-RV11 – fragment of the RNA polymerase (2a) gene, and primers 3a780F-CP50R – RNA3 noncoding intergenic region. Products obtained after amplification were resolved by electrophoresis in 1.2% agarose gel, stained with ethidium bromide and visualised on UV transilluminator.

Sequencing and computer analysis

RT-PCR products containing the coat protein gene, obtained in reaction with CPTALL5-CPTALL3 primers, were diluted 1:1000 (v/v) in distilled water and reamplified using FastStart Taq DNA Polymerase (Roche Diagnostics, Poland). After electrophoresis in 1.2% agarose gel, bands containing viral cDNA were cut from the gel, purified using the QIAquick PCR Purification Kit (Qiagen, Syngen, Poland) and then sequenced in the AbiPrism 3100 Genetic Analyzer apparatus (Applied Biosystems, USA), at the Maria Skłodowska Memorial Cancer Center and Institute of Oncology, Warsaw, Poland. Sequencing was performed using the same primers as for the original PCR and primers CMV1-CMV2.

CP gene nucleotide (nt) and deduced amino acid (aa) sequences were analyzed using the Lasergene v.

7.1 software package (DNASTAR, USA). Consensus sequence was produced using the SeqMan program, while determination of the similarity level of analyzed sequences were done using the MegAlign program and the GeneDoc program (<http://www.psc.edu/biomed/genedoc> Nicholas et al., 1997). The comparison of obtained cDNA fragments with sequences available in GenBank was accomplished using BLAST service available at <http://www.ncbi.nlm.nih.gov:80/BLAST> (Altschul et al., 1997). The phylogenetic relationship was estimated by application of the neighbour-joining method and subsequent bootstrap analysis in the MEGA software v. 4.0.2 (Tamura et al., 2007). CP sequences of tested isolates were compared with analogous RNA3 sequence fragments of the CMV reference strains: Fny, Mf, Y from group SD, Ix and IA from group IB as well as Ly, Q and Trk7 from group II (Roossinck, 2002). Isolates of TAV (strain KC; acc. no. AJ237849) and PSV (strain ER; acc. no. U15730) were included as outgroups.

Restriction analysis of PCR products

The RT-PCR product obtained after amplification with RW8-RV11 primers was digested using *Mlu*I enzyme, whereas RT-PCR products obtained with CPTALL3-CPTALL5 primers were digested separately with *Eco*RI and *Hpa*II enzymes (Fermentas, Lithuania). The reactions were carried out at 37°C for 16 h, and were then followed by 8% polyacrylamide gel electrophoresis.

RESULTS

ELISA detection of CMV isolates

Results of CMV detection using DAS-ELISA with specific polyclonal antibodies are summarized in Table 1. Most of the CMV isolates reacted with all tested PABs. However, isolate P26 did not react with M antibodies, isolate Simp2 did not react with Cas PAB and isolate Porz didn't show a reaction with ToRS antibodies. The best ELISA results in terms of a wide range of specificity were obtained with Wic antibodies. They reacted with all virus isolates. For most of the CMV isolates, the Wic antibodies exhibited the highest A_{405} values among all compared IgGs. Seven isolates – Cas, CB, D, Imp, J, M and Wic, also showed a strong reaction with M, DTL and ToRS antibodies. Low absorbance values were observed for isolates Simp2 and WicDS in reaction with ToRS IgG, as well as for isolates Inz and WicDS with DTL antibody.

RT-PCR detection of CMV isolates

The suitability of the IC-RT-PCR method with six pairs of CMV-specific primers has been checked for 15 isolates of CMV maintained in *N. rustica*. Specific amplification products of 950 bp for CPTALL5-CPTALL3, 860 bp for M1-M2, 650 bp for RW8-RV11, 500 bp for CMV1-CMV2 and 300 bp for 3a780F-CP50R primer pairs were obtained for all tested isolates (Fig. 1a and data not shown). Additionally to the specific products of expected

sizes, extra bands appeared in some experiments. These extra bands appeared especially after amplification of CMV templates using primers D3F-D3R (Fig. 1b).

To evaluate the suitability of three nucleic acid preparation methods (IC, SC and RN) for CMV diagnostics a dilution series of extracts (infected : healthy) were processed simultaneously. These extracts were tested with the primer pair CMV1-CMV2. The results of our experiments showed that application of each of the extraction protocols allowed isolation of viral nucleic acids suitable for enzymatic amplification. Visible bands were observed even for samples diluted 10^6 times (Fig. 2). A single product (~500 bp) was obtained in SC-RT-PCR and RN-RT-PCR (Fig. 2 b, c), while electrophoresis of IC-RT-PCR products revealed a specific band accompanied by an additional product of a size > 600 bp (Fig. 2 a).

Comparison of sensitivity and specificity of ELISA and RT-PCR

The sensitivity of ELISA and IC-RT-PCR was compared in the experiment with a tenfold serial dilution (down to 10^{-6}) of infected tobacco extract in healthy plant extract (1:50 w/v). Samples containing CMV were ELISA-positive only in undiluted preparations or in preparations diluted not more than 100 times, in assays with most of the tested PABs. Only with Wic antibodies was the virus detected in samples diluted 10^{-2} - 10^{-3} (Fig. 3). The sensitivity of IC-RT-PCR was much

Table 1. Detection of CMV isolates using DAS-ELISA with five sets of polyclonal antibodies

CMV isolate	Polyclonal antibodies				
	M	Wic	DTL	ToRS	Cas
Cas	+++	+++	++	++	+
CB	+++	+++	++	++	+++
D	++	+++	+++	+++	+++
Dn	+++	+++	++	++	++
Del	+++	+++	+	++	+
Imp	++	+++	++	+++	+++
Inz	++	+++	+	++	++
J	+++	+++	+++	+++	+++
M	+++	+++	++	++	+++
Og	++	+++	+	+++	+
P26	-	++	+	+	+
Porz	++	+++	++	-	+
Simp2	++	+++	+	+	-
Wic	++	+++	++	+++	+++
WicDS	+	+++	+	+	+
TAV	-	-	-	-	-
PSV	-	-	±	±	±
N.r.	-	-	-	-	-

ELISA results were scored as: +++ for A₄₀₅ values greater than 0.8, ++ for measurements of 0.4 to 0.8, + for measurements of 0.2 to 0.4, ± for values ranging from 0.1 to 0.19 and - for values lower than 0.1. TAV – tomato aspermy virus, PSV – peanut stunt virus, N.r. – healthy *N. rustica* plant.

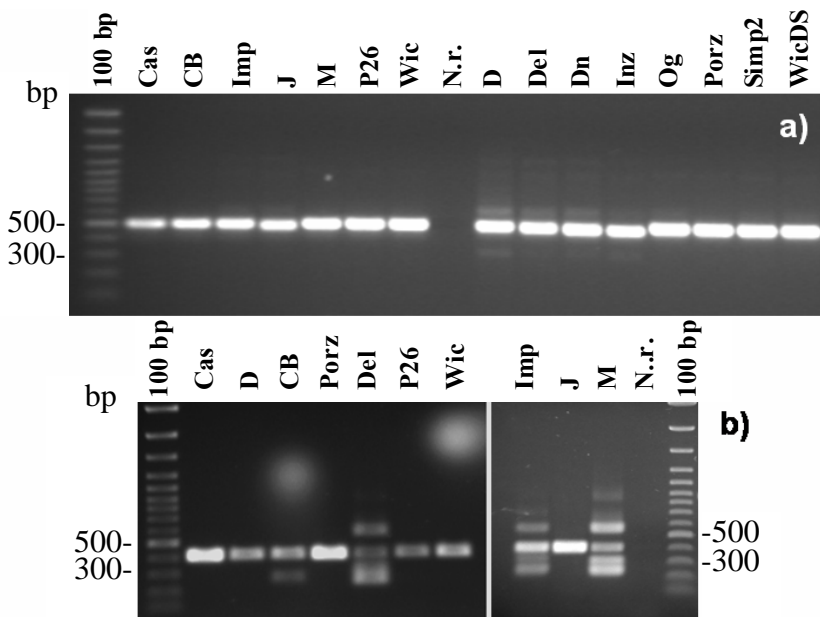


Figure 1. *Cucurbit mosaic virus* detection by IC-RT-PCR with CMV-specific primers: a) CMV1-CMV2, b) D3F-D3R 100 bp – GeneRuler 100 bp DNA Ladder, N.r. – healthy *N. rustica* plant

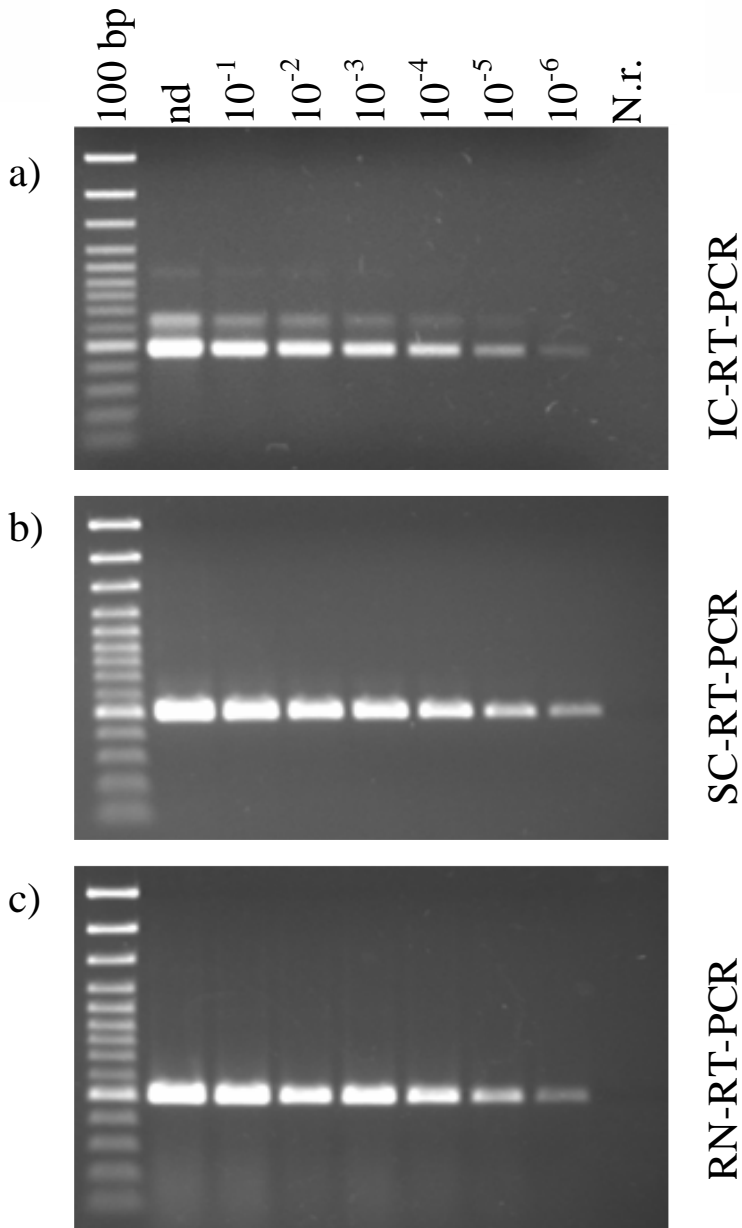


Figure 2. Sensitivity of CMV detection (isolate D) using IC-RT-PCR, SC-RT-PCR and RN-RT-PCR. nd – not diluted, 10⁻¹-10⁻⁶ tenfold serial dilution of CMV-infected plant extract in healthy plant extract; 100 bp – GeneRuler 100 bp DNA Ladder; N.r. – healthy *N. rustica* plant

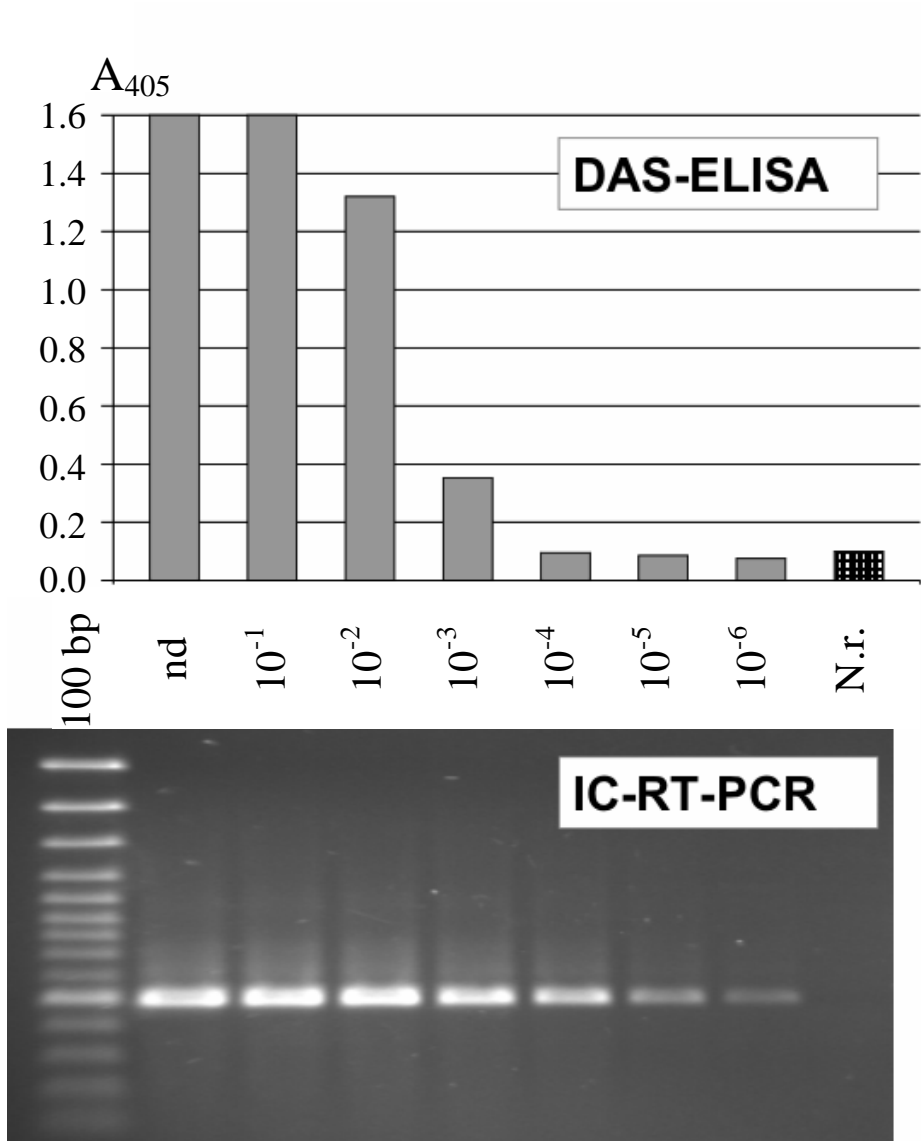


Figure 3. Sensitivity of CMV detection (isolate WicDS) using DAS-ELISA (PAb Wic) and IC-RT-PCR (primers CMV1-CMV2) methods. nd- undiluted sample; 10^{-1} - 10^{-6} – tenfold serial dilution of CMV-infected tissue extract in healthy *N. rustica* leaf extract; 100 bp – GeneRuler 100 bp DNA Ladder; N.r. – healthy *N. rustica* plant

higher than the sensitivity of DAS-ELISA – all tested virus isolates were detected in an extract of CMV-infected plants diluted at least 10^6 times.

CMV grouping

The suitability of DAS-ELISA with monoclonal antibodies and the analysis of CP gene sequence for CMV grouping was compared. For selected isolates, the usefulness of RT-PCR-RFLP analysis for differentiation of CMV groups was checked additionally.

On the basis of the reaction with CMV-I monoclonal antibodies, isolates Cas, CB J and M were classified as members of group I. Isolates D, Del, Dn, Imp, Inz, Og, Porz, Simp2, Wic and WicDS reacted only with CMV-II monoclonals, thus they were classified as group II of CMV. Isolate P26 did not react with either of the monoclonal antibodies used.

The identity between coat protein gene sequences of isolates D, Del, Imp, P26, Porz, Wic and WicDS, and sequences of other CMV group II isolates deposited in GenBank were greater than 98% at both - nucleotide and amino acids levels. Only from 76.6% to 78.2% nucleotide sequence identity was observed between those seven isolates and four other tested isolates (Cas, CB, M and J) and less than 73% identity with group I reference strains of CMV. At the amino acid level the identity of those seven isolates with group IA and IB isolates, ranged from 81% to 83%. Sequences of isolates Cas, CB, J and M

were most similar (identity 94% or more) to the CMV group IA isolates. Their identity with isolates belonging to groups IB and II was 81-91% and 78%, respectively.

Results of grouping of five isolates, based on RT-PCR-RFLP, were similar to those obtained with CP gene sequence analysis. After digestion of CPTALL5-CPTALL3 products with the enzyme *EcoRI*, different profiles were obtained for group IA and II isolates (Fig. 4a). Two bands of ~770 and 180 bp were observed for group II isolates (Imp, P-26 and Wic). Analyzed cDNA of group I isolates did not carry *EcoRI* site, thus only one band (~950 bp), corresponding to the size of the intact amplification product, was observed.

Analysis of *HpaII* digestion products allowed isolates to be classified into group IA or group II, as well as to differentiate further the tested group IA isolates (Fig. 4b). After electrophoresis of samples of the group II isolates, six DNA bands were observed (~250, 200, 180, 160, 130 and 30 bp; the smallest DNA fragment ~30 bp was not visible in the presented figure). For tested group I isolates, two restriction patterns were observed: bands of ~610 and 340 bp were obtained for M isolate, and four bands ~410, 190, 180 and 170 bp were present for isolate Cas.

Analysis of *MluI* enzymatic hydrolysis of the RW8-RV11 product allowed identification of group IA and group II isolates, as well as further differentiation of tested group II isolates (Fig. 4c). Two different restriction patterns were generated

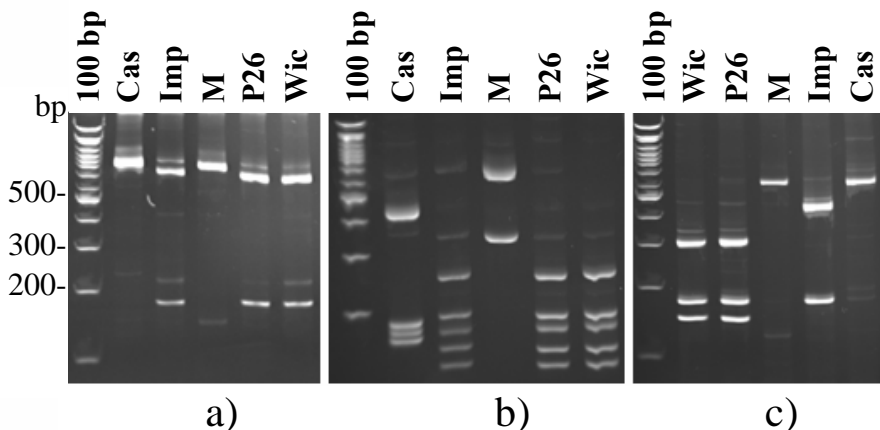


Figure 4. Identification of CMV isolates based on RFLP analysis of: a) CPTALL5-CPTALL3 amplification products digested with *EcoRI*, b) CPTALL5-CPTALL3 products digested with *HpaII*, c) RW8-RV11 products digested with *MluI*; 100 bp – GeneRuler 100 bp DNA Ladder

for group II isolates: bands of ~470 and 180 bp were present for isolate Imp, whereas for isolates P26 and Wic two bands ~320 bp and 150 bp appeared instead of a 470 bp product. Isolates Cas and M (group IA) did not carry *MluI* site.

DISCUSSION

There is a strong necessity for the use of highly sensitive methods to detect and differentiate between the CMV isolates. This necessity has grown with the tendency for global plant material exchanges and increasingly stringent plant material certification regulations. The best sensitivity is achieved using PCR-based methods (Wylie et al., 1993; de Blas et al., 1994; Hu et al., 1995; Raj et al., 2002). In many cases CMV detection by ELISA also gives satisfac-

tory results since the virus is usually found in high concentrations in naturally infected samples (Anonymous, 1998).

The quality and specificity of antibodies used for the preparation of ELISA reagents is critical for obtaining reliable results. Problems with CMV detection using PABs were reported by Hu et al. (1995), Raj et al. (2002) and Bashir et al. (2006). Some authors have suggested that application of monoclonal antibodies might increase the test specificity and sensitivity (Haase et al., 1989; Porta et al., 1989). On the other hand, other authors reported that the high specificity of MAb to particular CMV isolates might result in a lack of reaction with 'atypical' isolates showing relatively small differences in CP amino acid content and/or epitope conformation (Bashir et al.,

2006). Among CMV-specific polyclonal antibodies tested in our study, two out of five PABs allowed detection of all tested CMV isolates, whereas three other PABs did not react with one out of fifteen virus isolates. For each isolate, however, at least one of any two antibodies tested was able to detect it. Those results indicated that CMV detection by ELISA may be reliable only when at least two different polyclonals are used simultaneously.

IC-RT-PCR technique has been previously described as an efficient method of CMV detection by Varveri and Boutsika (1999), Yamaguchi et al. (2005) and Yu et al. (2005). In our experiment IC-RT-PCR allowed identification of all tested isolates, including those which were not detected in ELISA with some PABs. We did not observe differences in specificity of RT-PCR when different sets of primers were used. However, in some isolate-primer combinations additional non-specific amplification products were observed together with the specific cDNA bands. Since we speculated that those problems could be related to the immunocapture extraction procedure, we compared silicacapture and RNeasy protocols in parallel experiments. SC and RN methods allowed the isolation of good quality templates. During amplification this generated only specific cDNA products of expected size. Satisfactory results of RN- and SC-RT-PCR indicated that problems observed after IC-RT-PCR were not due to low specificity of primers or conditions

of amplification. They could be the result of the specific properties of plant extracts or the protocol used for coating and washing reaction tubes in our experiments. Unspecific IC-RT-PCR products were observed more frequently for naturally infected plants than for experimentally inoculated herbaceous plants, as well as for undiluted samples. Therefore, IC protocol may require further optimization.

Three assays were applied to differentiate and classify tested CMV isolates. Preliminary grouping was achieved using DAS-ELISA with monoclonal antibodies. In our experiment this assay enabled us to classify 14 out of 15 tested CMV isolates. One isolate – P26, could not be classified due to lack of reaction with both monoclonals used. High usefulness of MABs for grouping of CMV isolates was reported by Haase et al. (1989), Porta et al. (1989) and Anonymous (1998).

A detailed classification was achieved based on the comparison of coat protein nucleotide and amino acids sequences. In the CMV classification scheme, proposed by Palukaitis and Zaitlin (1997), CMV isolates that differ only 2-3% in their CP sequences are considered to be the members of the same group, whereas isolates with sequences divergence levels of 7-12% or more should be classified as members of different groups. According to this scheme isolates Cas, CB, J and M were put into group IA, whereas isolates D, Del, Imp, P26, Porz, Wic and WicDS were put into group II of

CMV. The results were confirmed by phylogenetic analysis.

Another method of CMV grouping was RFLP analysis of RT-PCR amplified products. Analysis of the restriction patterns of products digested with *EcoRI*, confirmed serotyping by monoclonal antibodies and allowed preliminary classification of tested isolates into group I or II. Our results of *EcoRI* digestions were similar to those described by Wylie et al. (1993). Classification of Cas and M isolates to group IA was achieved by RFLP analysis of RNA2 sequence fragments. Restriction profiles obtained in that experiment correlated with profiles of group IA isolates described by Finetti Sialer et al. (1999). They also matched with the computer-calculated pattern of the CMV group IA reference strains. The problematic P26 isolate that did not react in ELISA with MAbs showed a restriction pattern similar to the profiles of other CMV group II isolates.

In our experiment the classification of five CMV isolates using RFLP correlated with identification that was obtained using other methods. Some other authors suggested, however, that RFLP analysis needs to be treated with caution when used for CMV characterization. They found some group I isolates that generated a restriction pattern which could be confused with the profile of group II isolates (Varveri and Boutsika, 1999; Deyong et al., 2005).

Our results demonstrated that both serological and nucleic acids-based methods enabled effective

detection and identification of 15 CMV isolates found in different plant species in Poland. RT-PCR was, as expected, at least 100 times more sensitive than DAS-ELISA, although for routine detection and identification of CMV isolates ELISA with PABs or MAbs generally provided satisfactory results. This method was also more convenient and practical than RT-PCR for testing large numbers of samples. PCR should be used for verification of inconclusive ELISA results, in the cases when the virus is expected to appear at a very low concentration in the host plant, as well as when detailed CMV grouping is needed.

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PORÓWNANIE METODY ELISA I RT-PCR DO WYKRYWANIA I IDENTYFIKACJI IZOLATÓW WIRUSA MOZAIKI OGÓRKA (CMV) PORAŻAJĄCYCH ROŚLINY UPRAWNE W POLSCE

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S T R E S Z C Z E N I E

Porównano przydatność metody DAS-ELISA i RT-PCR do wykrywania 15 izolatów wirusa mozaiki ogórka (CMV). Efektywność wykrywania wirusa metodą ELISA zależała od rodzaju przeciwciał użytych do testu: przeciwciała Wic i DTL umożliwiły wykrycie wszystkich badanych izolatów, podczas gdy przeciwciała M nie reagowały z izolatem P26, przeciwciała ToRS nie wykrywały izolatu Porz, natomiast przeciwciała Cas nie reagowały z izolatem Simp2. Wszystkie badane izolaty wirusa wykrywano za pomocą metody RT-PCR. Porównano trzy metody przygotowania matryc do amplifikacji kwasów nukleinowych: wychwytywanie cząstek wirusa z użyciem przeciwciał (immunocapture, IC), adsorpcję kwasów nukleinowych na żelu krzemionkowym (silicacapture, SC) oraz izolację RNA z wykorzystaniem komercyjnego zestawu (RN). Badania wykazały, że zastosowanie do amplifikacji preparatów przygotowanych każdą z wymienionych metod umożliwia wykrycie CMV. Identyfikację i grupowanie badanych izolatów wykonano za pomocą: metody ELISA z użyciem grupowo-specyficznych przeciwciał monoklonalnych, analizy filogenetycznej sekwencji genu białka płaszczka oraz analizy restrykcyjnej zamplifikowanych fragmentów RNA2 i RNA3 wirusa poddawanych działaniu enzymów restrykcyjnych *EcoRI*, *HpaII* i *MluI*. Zastosowane przeciwciała monoklonalne umożliwiły wstępną klasyfikację 14 izolatów do grupy I lub II, jednakże nie reagowały one z izolatem P26. Analiza sekwencji genu białka płaszczka umożliwiła identyfikację wszystkich badanych izolatów oraz przyporządkowanie ich do grupy IA lub II. Wyniki klasyfikacji izolatów uzyskane po analizie restrykcyjnej były zgodne z wynikami grupowania otrzymanymi w analizie filogenetycznej.

Słowa kluczowe: wirus mozaiki ogórka, DAS-ELISA, RT-PCR, RFLP, analiza sekwencji