Agronomy Research **4**(Special issue), 471–474, 2006

The use RT-PCR for detection of viruses infecting cucumber

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Abstract. Cucumber plants exhibiting virus-like disease symptoms were collected in various gardens and greenhouses in Lithuania. Viral diseases were expressed by symptoms of mosaic-mottling, yellow ringspots and vein necrosis of leaves. Growth and fruiting of plants were reduced. Results of the identification of viruses detected in cucumbers by the RT-PCR technique are presented here. Identification of causal agents was based on cDNA amplified product size in PCRs using virus-specific oligonucleotides. From cucumbers with various virus-like symptoms 400, 499 and 300 bp DNA fragments were amplified. They are characteristic for *Cucumber mosaic virus* (CMV)*, Tomato ringspot virus* (ToRSV) and *Tobacco necrosis virus* (TNV)*,* respectively, and confirm cucumber infection by these viruses.

Key words: cucumber, virus, detection, RT-PCR

INTRODUCTION

About twenty economically important viruses were detected in cucumber (*Cucumis sativus* L.) crops (Brunt et al., 1996). *Cucumber mosaic cucumovirus* (CMV) is reported to infect 1287 plant species. CMV was detected in leguminous, ornamental (Staniulis et al., 1977) and tomato (Zitikaite, 1999) plants in Lithuania. *Tomato ringspot nepovirus* (ToRSV) naturally affects 518 plant species (Edwardson & Christie, 1997). ToRSV is readily transmissible by the nemathode in soil. A high incidence of ToRSV infection was also revealed in ornamental plants in Lithuania (Samuitienė & Navalinskienė, 2001). *Tobacco necrosis necrovirus* (TNV), which is preserved in infected plant parts in the soil, and transmitted by soil fungus, often causes cucumber necrosis. It can infect 298 plant species (Kassanis, 1970; Šutic et al., 1999), and was detected in both horticultural (Staniulis, 2003; Zitikaitė et al., 2005) and ornamental (Navalinskienė, 1994) plants in Lithuania. Preliminary characterization of these viruses from cucumbers was performed by mechanical transmission in test plants of *Nicotiana glutinosa* and *N. rustica*. The virus detection by traditional methods is time consuming. A combination of virus-specific primers and thermostable DNA polymerase were used to amplify the target sequence, through repeated cycles of denaturation, reannealing, and DNA synthesis at high temperatures (Saiki et al., 1988). For the accurate detection of viruses in cucumbers the nucleic acid-based PCR method was used.

The aim of this investigation was detection of causal agents for virus-infected cucumber crops, using the RT-PCR assay.

MATERIALS AND METHODS

The material for virus detection was collected from cucumbers expressing viral symptoms on leaves or fruits. Nucleic acids for identification of CMV and ToRSV were extracted from 10 samples using the small-scale procedure as proposed for extraction of nucleic acids from woody plants (Zhang et al., 1998). Total RNA extraction from cucumber samples for detection of infection with TNV was carried out according to the instruction of "QuickPrep™ total RNA Extraction Kit for the direct isolation of total RNA from most eukaryotic tissues or cells" (Amersham Biosciences, UK). Primer pairs used in RT-PCRs for CMV (de Blass et al., 1994), ToRSV (Griesbach, 1995) and TNV-A „Nebraska" isolate (MEDLINE, Acc. No 93393432) were designed according to viral sequence information. Pellets of total RNA were resuspended in the solution containing RNAse inhibitor, primer Reverse and PCR water and incubated at 70ºC for 10 min. For the first strand cDNA synthesis the RNA pellet solutions were added to the mixture containing 5x Reaction buffer, RNAse inhibitor, dNTPmix and RevertAid™M-MuLV RT (MBI Fermentas, Lithuania). The first strand cDNA synthesis was carried out at 37ºC for 60 min and at 70ºC for 10 min. DNA amplifications were performed in reaction mixtures containing dNTPmix, both primers, 10xPCR buffer with $MgCl_2$ and recombinant *Taq* polymerase (MBI Fermentas) using Eppendorf Mastercycler Personal. PCRs were carried out for 40 cycles using the following parameters: 1 min at 94ºC (4 min for the first cycle), 2 min at 52ºC and primers extension for 2 min (10 min in the final cycle) at 72ºC. DNA fragment size standard was Φx174DNA/BsuRI(*Hae*III) digest (MBI Fermentas) (from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp). Resulting PCR products were analysed by electrophoresis through 5% polyacrylamide gel, stained with EB, and DNA bands were visualized under UV light.

RESULTS AND DISCUSSION

RT-PCR for detection of CMV, ToRSV and TNV in cucumbers was successfully used. CMV was detected in outdoor cucumber plants of cultivars Alfa, Mirabelle and Movir. The symptoms caused by this virus in cucumbers varied, exhibiting leaf light green mosaic and decreased leaves, shortened stem internodes, and slow growth. These symptoms are characteristic to members of *Cucumoviruses* (Kaper & Waterworth, 1981). Specific bands of RT-PCR products were observed at the position corresponding to the expected size of the DNA amplification products of about 400 bp for CMV (Fig. 1).

ToRSV isolates from cucumbers of cultivars Restima, Ventura and Polan with symptoms of yellow ringspot were investigated. The virus properties corresponded preliminary to ToRSV and to other *Nepoviruses* (Murant, 1981). The virus-specific primers amplified DNA fragment of 499 bp (Fig. 2) from the coat protein gene of ToRSV from infected cucumbers in RT-PCR.

Samples of cucumbers with TNV disease showed yellow spots on young leaves, and vein necrosis and necrotic spots on older leaves. Fruits were small, deformed, yellow mottled and severely puckered. Symptoms were present on about 2-3% of the cucumber plants. Specific PCR products of TNV were obtained at a position of approximately 300 bp (Fig. 3).

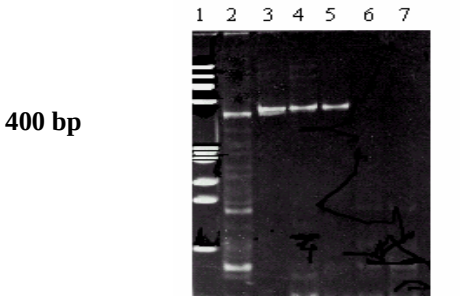

Fig. 1. PCR products of CMV: l, DNA Ladder, 2, 3, cucumber samples; 4, 5, tomato samples; 6, healthy cucumber plant; 7, water control.

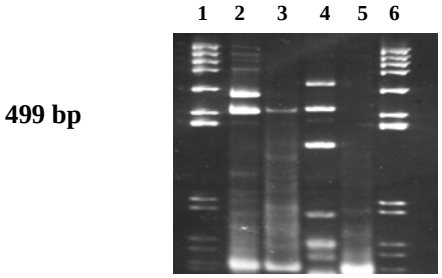

Fig. 2. PCR products of ToRSV: 1, 6, DNA Ladder; 2, cucumber sample; 3, tomato; 4, iris; 5, water control.

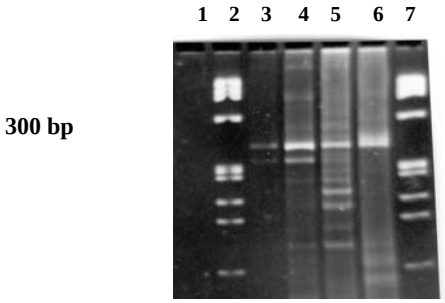

Fig. 3. PCR products of TNV: 1, water control; 2, 7, DNA Ladder; 3, 4, 5, 6, cucumber samples

The primer pairs designed for these viruses on the basis of published sequences specifically amplified cDNA templates in RT-PCRs and confirmed infection of viruses from cucumber samples.

CONCLUSION

The results obtained in this study show the successful use of RT-PCR as a rapid assay for direct detection of viruses in infected tissues of cucumber crop.

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