Real-time PCR in diagnostics of plant viruses

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Introduction

For identification of plant viruses ELISA, immuno-serological electron microscopy, test plants and RT-PCR or PCR are frequently in use. Recently, a more sensitive and specific method real-time PCR was developed for diagnostics of certain plant viruses. Real-time PCR can be used for determination of different viruses as well as for differentiation between related strains of viruses. The method can also be used for detection of low concentrations of plant viruses in various samples such as environmental waters, growth substrates, and seeds.

Material and methods

In real-time PCR (TaqMan) reactions used for detection of viral presence in the plant material the internal controls 18S (commercially available, ABI) or COX (Boonham, personal communication) were included in the assays in order to control the RNA extraction procedure. Reverse transcription step was performed separately or one-step real-time RT-PCR was performed and compared with two-step assay.

Real-time PCR in diagnostics of plant viruses at NIB

Detection and differentiation of two tospoviruses

Table 1: Detection of CSNV and TSWV with real time PCR

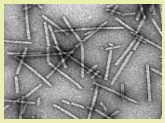
Samples of *Chrysanthemum* plants	CSNV results	TSWV results
D1000/01 (TSWV)	-	+
D800/01 (CSNV)	+	-
D645/01 (TSWV and CSNV)	+	+
Negative control	-	-

(-) negative; (+) positive

Chrysanthemum stem necrosis virus (CSNV) serologically cross-reacts with many commercially available antisera against *Tomato spotted wilt virus* (TSWV) in ELISA assay. Symptoms on *Chrysanthemum* are very similar in both, CSNV and TSWV (Ravnikar et al., 2004).

In our laboratory, real-time PCR was developed for identification of *Chrysanthemum stem necrosis virus* (CSNV). The method for detection of serologically closely related Tospovirus *Tomato spotted wilt virus* (TSWV) (Boonham et al., 2002) was successfully implemented in the diagnostic procedures.

Real-time PCR used alone or in combination with other methods is a way for efficient and reliable detection and differentiation of this two tospoviruses.



Detection of low concentrations of plant viruses in plants, environmental waters and growth substrates

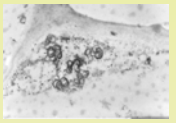


Table 2: Detection of ToMV with real time PCR and ELISA

Method	ToMV concentration (mg ml^{-1})	LOQ	LOD
real time PCR	$4{,}2 \times 10^{-3}$	■	○
	$4{,}2 \times 10^{-4}$	■	○
	$4{,}2 \times 10^{-5}$	■	○
	$4{,}2 \times 10^{-6}$	■	○
	$4{,}2 \times 10^{-7}$	■	○
	$4{,}2 \times 10^{-8}$	■	○
	$4{,}2 \times 10^{-9}$	NR	○
	$4{,}2 \times 10^{-10}$	NR	○
	$4{,}2 \times 10^{-11}$	negative	negative
ELISA	$0{,}28 \times 10^{-3}$	■	○
	$0{,}93 \times 10^{-4}$	■	○
	$3{,}1 \times 10^{-5}$	■	○
	$3{,}4 \times 10^{-6}$	negative	negative

LOQ – limit of quantification; LOD – limit of detection; ■ – ToMV reliably detected and quantified in the sample; ○ – ToMV detected in the sample; NR – ToMV not reliably quantified in the sample

Table 3: Detection of virus PVY^{NTN} with different methods in potato (*Solanum tuberosum*) cv. Igor

dpi / samples	real time PCR	ELISA	electron microscopy
5 dpi / inoculated leaves	+	+	+
5 dpi / upper non-inoculated leaves	+	-	-
14 dpi / upper non-inoculated leaves	+	+	+
14 dpi / newly developed leaves	+	+	+

(dpi) days post-inoculation; (-) negative; (+) positive

Real time PCR was also shown to be more sensitive than other methods in detection of *Potato virus* Y (PVY) in potato leaves (Mehle et al., 2004).

Concentration of plant viruses in irrigation waters and soil is usually below the sensitivity of frequently used detection methods such as ELISA.

Real-time PCR for identification of *Tomato mosaic virus* (ToMV) in tomato and tobacco plants, irrigation waters and soil was developed.

The method was shown to be 1000 times more sensitive than ELISA (Boben et al., 2006).

Literature

Boben J, Kramberger P, Petrovič N, Cankar K, Peterka M, Štrancar A, Ravnikar M. 2006. Detection of ToMV (Tomato mosaic virus) in irrigation waters. In: Dolenc Koce J. (ed.), Vodnik D. (ed.), Dermastia M. (ed.). 4th Slovenian Symposium on Plant Biology with International Participation, Ljubljana, September 12-15, 2006. Book of abstracts. Ljubljana: The Slovenian Society of Plant Physiology, 2006, 105-106.

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