Development of real-time PCR assay for *Chrysanthemum stem necrosis virus*

BOBEN, J.[1], ZUPANČIČ, M.[1], MAVRIČ, I.[1, 2], VOZELJ, N.[1], PETROVIČ, N.[1], RAVNIKAR, M.[1],

[1]National Institute of Biology, Department of Plant Physiology and Biotechnology, Večna pot 111, SI-1000 Ljubljana, Slovenia (jana.boben@nib.si)

[2]Current address: Agricultural Institute of Slovenia, Haquetova 17, SI-1000 Ljubljana, Slovenia

ABSTRACT

Chrysanthemum stem necrosis virus (CSNV) is a relatively new virus. It was first reported in Brasil in 1995 and later on in The Netherlands and in the United Kingdom. Survey of Tospoviruses in Slovenia, performed by national Plant protection service, revealed presence of CSNV in ornamental plants *Chrysanthemum* (Figure 1) and *Gerbera*. According to the symptoms, CSNV virus could not be distinguished from other tospoviruses. We proved that CSNV cross-reacts in ELISA with several commercially available antisera against TSWV (*Tomato spotted wilt virus*). Therefore RT-PCR was developed for diagnosis of CSNV (Ravnikar et al., 2003). We upgraded the detection system with real-time PCR assay. Specificity and cross-reactivity of designed primers and probe were tested on two CSNV isolates from Slovenia, on related tospoviruses (TSWV and INSV - *Impatiens necrotic spot virus*), and on various host plants. Our data shows that real - time PCR can reliably distinguish between CSNV and TSWV.

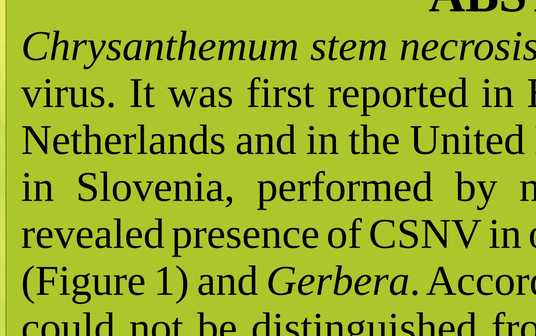

Figure 1: *Chrysanthemum* showing CSNV symptoms.

MATERIALS AND METHODS

Plant material:

· CSNV infected *Chrysanthemum*, *Gerbera*, and *Nicotiana benthamiana* - (leaves)

· TSWV infected *Chrysanthemum* and *Nicotiana rustica* - (leaves)

· *Chrysanthemum* infected with CSNV and TSWV (leaves, stem, roots)

· INSV infeceted *Nicotiana benthamiana* (leaves)

RNA was extracted using RNeasy Plant Mini Kit (Qiagen).

RT-PCR:

Two sets of primers were designed to detect CSNV and one set of primers was used to detect TSWV (Weekes, 1996).

Real-time PCR:

CSNV specific primers and MGB probe were designed using Primer Express® software (Applied Biosystems). TSWV primers and probe were also used in test (Boonham et al., 2002). COX primers and probe presented internal control of RNA extraction (Boonham, personal communication). Data was analysed using SDS 2.1 software (Applied Biosystems).

RESULTS AND DISCUSSION

Designed primers and probe specifically detected plants infected with CSNV. We could not detect any cross-reactivity with plants infected with other Tospoviruses tested (TSWV and INSV). Results also show that CSNV can be deteced by real-time PCR method in different host plants (*Chrysanthemum*, *Gerbera*, and *Nicotiana*) (Table 1).

Table 1: Results of real-time PCR using two sets of specific primers and probes. Designed primers and MGB probe show specific reactivity in CSNV infected plants and no cross-reactivity with other Tospoviruses regardless of the plant tested.

plant	CSNV probe	TSWV probe
CSNV infected *Chrysanthemum*	+	-
CSNV infected *Gerbera*	+	-
CSNV infected *N. benthamiana*	+	-
TSWV infected *Chrysanthemum*	-	+
TSWV infected *N. rustica*	-	+
INSV infected *N. benthamiana*	-	-

RT-PCR results in *Chrysanthemum* plant with mixed CSNV and TSWV infection showed presence of both TSWV and CSNV in leaves and stems, whereas only TSWV could be detected in roots (Figure 2). Intensity of CSNV specific band was higher in leaves whereas TSWV specific band was more intensive in roots.

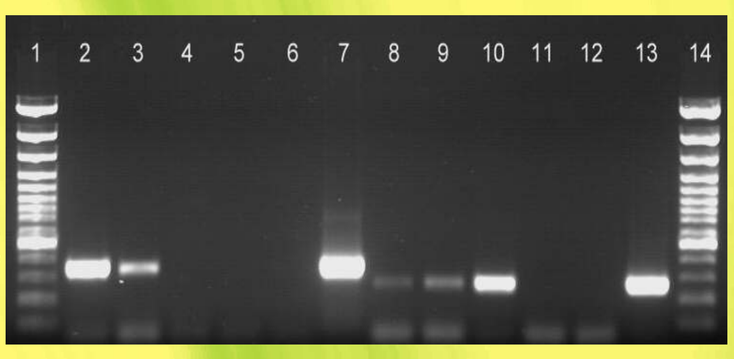

Figure 2: RT-PCR on CSNV and TSWV infected *Chrysanthemum*. 2-7 CSNV specific primers, 8 - 13 TSWV specific primers, 1, 14 DNA ladder 100 bp; 2 and 8 - leaves, 3 and 9 - stem, 4 and 10 - roots, 5 and 11 negative controls, 6 and 12 water control, 7 CSNV and 13 TSWV controls of reaction.

Real-time PCR results confirm RT-PCR results, they also show higher concentrations of CSNV virus in leaves and higher concentration of TSWV virus in roots of the plant tested (Figure 2 and Table 2).

Table 2: Combined results of RT-PCR and real-time PCR on different parts of *Chrysanthemum* plant with mixed infection.

virus	method	LEAVES	STEM	ROOTS
CSNV	RT-PCR	+	+	-
	real-time PCR (Ct)	24.8	27.8	ND
TSWV	RT-PCR	+	+	+
	real-time PCR (Ct)	28.1	28	24.8

REFERENCES

· Weekes, R. J., Mumford, R. A., Barker, I., Wood, K. R. 1996. Acta Horticulturae, 431, 159-166.

· Boonham, N., Smith, P., Walsh, K., Morris, J., Spence, N., Benison, J., Barker, I. 2002. The detection of Tomato spotted wilt virus (TSWV) in individual thrips vectors using real time fluorescent RT-PCR (TaqMan). Journal of Virological Methods, 101, 37-48.

· Alexandre, M. A. V., Rivas, E. B., Duarte, L. M. L., Okuyama, M. H., 1996. Serological survey of tospovirus on *Chrysanthemum* sp. Crops in Sao Paulo State, Brazil. Fitopatologia Brasileira 21: 80-84.

· Alexandre, M. A. V., Duarte, L. M. L. Rivas, E. B., Chagas, C. M. 1999. Mixed infection by *Tospovirus* species in ornamental crops in Sao Paulo State, Brazil. Summa Phytopathologica, 25: 353-356.

· Verhoeven, J. Th. J., Roenhorst W., Cortes, I., Peters, D. 1996. Detection of a novel Tospovirus in Chrysanthemum. Acta Horticulturae, 432: 44-51.
Plant Health Newsletter. 2003. DEFRA Department for Environment, Food & Rural Affairs. January, 2003. PH/004

· Ravnikar, M., Vozelj, N., Mavrič, I., Švigelj, S. D., Zupančič, M., Petrovič, N. 2003. Detection of *Chrysanthemum stem necrosis virus* and *Tomato spotted wilt virus* in Chrysanthemum. Abstracts of offered papers, 8th International Congress of Plant Biology, 2 7 February 2003, Christchurch, New Zealand. P. 306.