APPLIED PLANT RESEARCH
WAGENINGEN UR

Molecular identification and detection of Tulip severe mosaic virus, a member of the family *Closteroviridae*

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Introduction

Virus infections in tulips (Tulipa L.) grown in the Netherlands were monitored and studied extensively in the past decades. Many of the observed viruses were characterized serologically and some of them also molecularly. The analysed viruses include the filamentous viruses, e.g.Tulip breaking virus (TBV), Lily symptomless virus (LSV), Tulip virus X (TVX) and Turnip mosaic virus (TuMV). Diagnostic tools based on ELISA or PCR became available for routine screening against these viruses. One of the few uncharacterized viruses in tulip today is the rare occurring Tulip severe mosaic virus (TSMV), a filamentous virus measuring 1600-2400 nm that causes severe mosaic symptoms in tulip (Figure 1).

Molecular identification

So far, no antiserum is available for the detection of TSMV and the only way to confirm TSMV infection is based on labour-intensive electron microscopical analysis of the particle morphology. We have developed a TSMV detection method based on the reverse transcription-polymerase chain reaction (RT-PCR) using degenerate primers targeted to a conserved region of the heat shock protein 70 gene (Hsp70) of viruses from the family *Closteroviridae*. PCR-amplification products were generated (±500 bp) and the sequences were phylogenetically analysed. (Figure 2) .

Figure 1: Symptoms caused by Tulip severe mosaic virus (TSMV) on tulip.

Results & Discussion

Alignments of these Hsp70 gene fragments showed that TSMV is closely related to *Plum bark necrosis stem pitting-associated virus* (PBNSPaV), *Apricot stem pitting-associated virus* (ASPaV) and *Pineapple mealybug wilt-associated virus 1* (PMWaV-1), all three belonging to the genus *Ampellovirus.* A set of TSMV specific primers was designed that specifically amplifies a 450 bp Hsp70 fragment of TSMV. The RT-PCR assay with these primers is now a suitable diagnostic tool for TSMV (Figure 3).

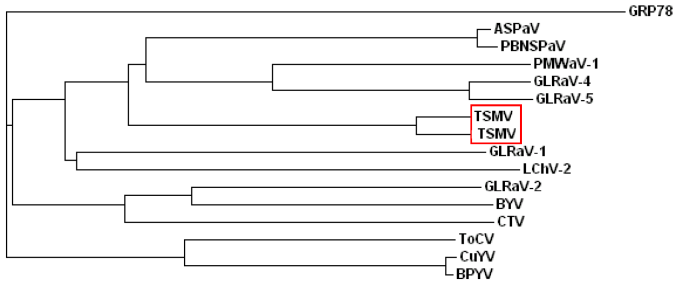

Figure 2: A phylogenetic tree generated from an alignment of the amino acid sequences between phosphate1 and connect1 motives of the HSP70 protein of two TSMV isolates and other viruses of the family *Closteroviridae*. Accession numbers of the sequences used are: *Apricot stem pitting-associated virus* (ASPav) CAC83741; *Plum bark necrosis stem pitting-associated virus* (PBNSPaV) AAF04602*; Pineapple mealybug wilt-associated virus 1* (PMWaV) AAL66711; *Grapevine leafroll-associated virus 4* (GLRaV-4) AAB96680; *Grapevine leafroll-associated virus 5* (GLRaV-5) AAK38608; *Little cherry virus 2* (LChV-2) AAP87788; *Grapevine leafroll-associated virus 2* (GLRaV-2); *Beet yellows virus* (BYV) CAA51858; *Citrus tristeza virus* (CTV) AAC59627; *Tomato chlorosis virus* (ToCV) AAD01790; *Cucumber yellows virus* (CYV) BAC66364; *Beet pseudo yellows virus* (BPYV) AAQ97386. Sequence of the GRP78 protein from tomato was used as outgroup.

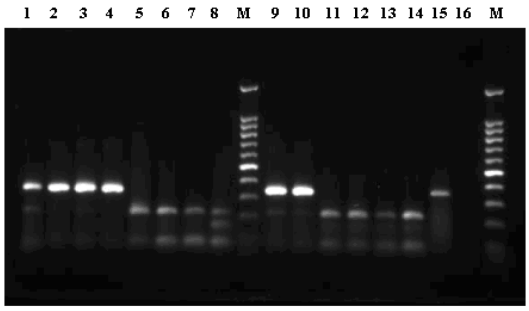

Figure 3: RT-PCR products amplified with specific primers for Tulip severe mosaic virus (TSMV). Lane 1-4 and 9, 10, 15: severe TSMV infected tulips samples (the presence of viruses was conformed by EM); lane 5-8: tulips infected with TuMV, TBV, TRV, TNV resp.; lane11-14: healthy tulips; lane 16: water (negative control). M; 100bp Ladder (Promega).water (negative control). M; 100bp Ladder (Promega).

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