# Rapid lateral flow tests, protein and nucleic acid assay, for Tulip Virus X detection in plants

Marjo Koets<sup>1</sup>, Jan Wichers<sup>1</sup>, Joop van Doorn<sup>2</sup>, Maarten de Kock<sup>2</sup> and Aart van Amerongen<sup>1</sup> Wageningen University and Research Centre, <sup>1</sup>Food & Biobased Research and <sup>2</sup>Applied Plant Research



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# Background

Tulip Virus X (TVX) is a pathogen for tulip. It is a filamentous, positive-stranded RNA virus, belonging to the family of Flexiviridae and the genus of Potex viruses. Its natural hosts are Tulipa species. Symptoms of infection comprise chlorotic or necrotic grey-brown streaking of leaves and streaks of intensified pigment (or of necrosis) in petals (Figure 1). Several mechanical transmission pathways for TVX have been identified during bulb production. During bulb storage, the dry bulb mite (Aceria tulipae) is the main vector for TVX. Preventive actions focused on early recognition and removal of virus-diseased plants are the best remedies to combat this virus. To support farmers and inspection services in early recognition of virusdiseased plants, we developed two methods that can be applied in or near the field, both based on the wellknown Lateral Flow ImmunoAssay (LFIA) principle.





**Figure 1.** Tulip showing greybrown streaking of leaves typical for TVX infection.

act of TVX contaminated

# **Objectives**

- To develop a simple and rapid method for screening of tulips for TVX contamination in field settings.
- A rapid and easy to perform nucleic acid based assay which can be used to detect the virus in symptom-free batches of tulips or to discriminate between viruses causing similar symptoms as TVX in tulip cultivars, e.g. Augusta virus and Tobacco Rattle Virus (TRV).

# Results

### Protein based assay

A specific polyclonal antiserum directed against the virus coat protein (CP), was used both as capture ligand on the nitrocellulose membrane and as detection ligand immobilised on the surface of carbon nanoparticles. Plant extracts were prepared from healthy and TVX-contaminated tulip leaves and directly applied to the LFIAs. Figure 2 shows a concentration range of extract diluted in assay buffer. A clear black line is visible with increasing line-intensity at higher volumes of extract (right set of membranes), indicating the presence of the virus in the samples. No non-specific lines were visible upon running the corresponding negative controles (left set of membranes).



**Figure 2.** LFIA test format. Left: a schematic view of the TVX-LFIA. Right: LFIA results of Tulip leave extracts. Carbon-conjugated antibodies against

Carbon-conjugated antibodies against TVX bind to the virus in the sample, a sandwich is formed with specific antibodies at the test line and a black line appears. Various volumes of extract, TVX-contaminated and TVX-free, were applied to the assays.

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Negative control: µl extract of healthy Tulip leaves



Wageningen UR Food & Biobased Research
 Biomolecular Sensing & Diagnostics
 P.O. Box 17, 6700 AA Wageningen
 Contact: aart.vanamerongen@wur.nl
 T + 31 (0)317 480164, M +31 (0)6 41361125
 http://www.fbr.wur.nl/uk

### Nucleic acid based assay

A double-labelled amplicon was sandwiched between an antidigoxigenin antibody on the membrane and neutravidin on carbon nanoparticles. The double-labelled amplicon was obtained by PCR amplification of TVX cDNA using 5'-end labelled primers (Table 1). The forward primer and reverse primer were labelled with biotin and digoxigenin, respectively (Figure 3).

 Table 1. Primer sequences for specific amplification of a coding region of TVX coat protein.



Figure 3. Assay principle of the TVX Nucleic Acid Lateral Flow ImmunoAssay.

The PCR product was diluted in assay buffer and directly applied to the 'Nucleic Acid LFIA' (NALFIA). Figure 4 shows the results of the TVX amplification detected by gel electrophoresis (left picture) and by NALFIA (right picture). TVX-specific DNA fragments were amplified in the samples containing TVX cDNA. PCR material (1  $\mu$ L) was applied to the NALFIA strips and the labelled amplicons were specifically sandwiched between the corresponding antibody on the membrane and neutravidin on the carbon nanoparticles. No visible lines were observed with antibodies specific for other labels.



Figure 4. Results obtained with the NALFIA. PCR material from amplified virus cDNA (1 and 3) and a without-template control (2 and 4) were applied to an agarose gel and to NALFIA membranes.

### Conclusions

- We demonstrate specific detection of TVX using rapid protein and DNA based immunoassays.
- For direct on site application the LFIA can be applied using plant juice of suspected tulips. However, although the user needs an extra step (PCR amplification) the NALFIA is more specific and sensitive and might even detect TVX in symptomless (latent infected) tulips.
- The TVX specific (NA)LFIAs can support the decision of tulip growers to remove suspected plant, thus reducing the chance of further spread of the virus.

Wageningen UR Applied Plant Research P.O. Box 85, 2160 AB Lisse Contact: joop.vandoorn@wur.nl, T + 31 (0)317 462149 http://www.pri.wur.nl