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

Marjo Koets¹, Jan Wichers¹, Joop van Doorn², Maarten de Kock² and Aart van Amerongen¹
Wageningen University and Research Centre, ¹Food & Biobased Research and ²Applied Plant Research

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 (Aceroa 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 virus-diseased plants, we developed two methods that can be applied in or near the field, both based on the well-known Lateral Flow ImmunoAssay (LFIA) principle.

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 LFIA. 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 controls (left set of membranes).

Nucleic acid based assay
A double-labelled amplicon was sandwiched between an anti-digoxigenin 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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5'-3')</th>
<th>Target</th>
<th>Amplicon</th>
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<tbody>
<tr>
<td>TVX-for-biotin</td>
<td>bca cac cgt gtt</td>
<td>Coat Protein TVX (CP)</td>
<td>453 bp</td>
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<tr>
<td>TVX-rev-digoxigenin</td>
<td>tgg gcc acg ctg gcc ggg gta gtt</td>
<td></td>
<td>&lt;53 bp</td>
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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 µL) 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 (N)ALFIAs can support the decision of tulip growers to remove suspected plant, thus reducing the chance of further spread of the virus.