Generic RT-PCR tests for detection and identification of Tospoviruses

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Genus Tospovirus

Family Bunyaviridae

RNA segments
L: 8.8 kb
M: 4.8 kb
S: 2.9 kb
Species demarcation criteria Tospoviruses (ICTV)

- Vector specificity
- Plant host range
- Serological relationship Nucleocapsid (N) protein
- N-protein sequence less than 90% aa identity (S segment)
Impact of Tospoviruses

- So far 29 species described
- Infect a broad range of plant species
- Cause serious damage in important crops worldwide
- Transmitted by various difficult to control thrips species
- Natural resistance limited
- EU-regulated tospoviruses
  - Chrysanthemum stem necrosis virus (CSNV)
  - Tomato spotted wilt virus (TSWV)
  - Impatiens necrotic spot virus (INSV)
Diagnosis of Tospoviruses

- Indicator plants - *Nicotiana benthamiana*
  - Detection by symptoms
  - Identification not possible

- Serological tests – ELISA
  - Cross reactions between species

- Molecular tests - RT-PCR and sequence analysis
  - Developed for a small range of tospoviruses
  - Poor knowledge about specificity
Objective

- Generic RT-PCR test(s) for detection of Tospoviruses
- Identification by sequencing and analysis of amplicons
Tospovirus species

- American clade 1
  - Tomato chlorotic spot virus (TCSV)
  - Groundnut ringspot virus (SRSV)
  - Pepper necrotic spot virus (PNSV)
  - Alstroemeria necrotic streak virus (ANSV)
  - Tomato spotted wilt virus (TSWV)
  - Zucchini lethal chlorosis virus (ZLCV)
  - Chiysanthemum stem necrosis virus (CSNV)
  - Melon severe mosaic virus (MSMV)
  - Impatiens necrotic spot virus (INSV)
  - Soybean vein necrosis-associated virus (SVNvA)
  - Bean necrotic mosaic virus (BeNMV)
  - Iris yellow spot virus (YSV)
  - Tomato yellow ring virus (TYRV)
  - Polygonum ringspot virus (PoRSV)
  - Hippeastrum chlorotic ringspot virus (HCRV)
  - Watermelon bud necrosis virus (WBNV)
  - Groundnut bud necrosis virus (GBNV)
  - Watermelon silver mottle virus (WSMoV)
  - Cucumis chlorotic virus (CCv)
  - Mulberry vein banding associated virus (MVBvA)
  - Tomato necrotic spot virus (TNSV)
  - Calla lily chlorotic spot virus (CLSv)
  - Tomato zonate spot virus (TZZv)
  - Tomato necrotic ringspot virus (TNRv)
  - Pepper chlorotic spot virus (PCSV)
  - Melon yellow spot virus (MYSv)
  - Peanut chlorotic leaf-spot virus (PCFv)
  - Groundnut yellow spot virus (GYSv)
  - Lysianthus necrotic ringspot virus (LNRv)
  - Bunyamwera virus (BUNv)

- American clade 2

- Eurasian clade

- Asian clade 1

- Asian clade 2
Primer design

- Nucleotide sequences of 28 different species from GenBank: N gene and 5’ and 3’ non-coding trailer sequences (S segment)
- Multiple sequence alignment (MAFFT)
- Selection of (sub)clade specific conserved region(s)
- (Sub)clade specific amplicon length

**Diagram:**

- **AM-1**
  - UTR
  - N protein
  - Amplicon: ~760 bp
- **AM-2**
  - Amplicon: ~670 bp
- **EA**
  - Amplicon: ~780 bp
- **AS-1**
  - Amplicon: ~360 bp
- **AS-2**
  - Amplicon: ~510 bp
- **LNRV**
  - Amplicon: ~430 bp
## Isolates used for testing

<table>
<thead>
<tr>
<th>Species</th>
<th>Code</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCSV</td>
<td>BR-03</td>
<td>WUR</td>
</tr>
<tr>
<td>GRSV</td>
<td>SA-05</td>
<td>WUR</td>
</tr>
<tr>
<td>ANSV</td>
<td>Columbia</td>
<td>WUR</td>
</tr>
<tr>
<td>TSWV</td>
<td>BR-01</td>
<td>WUR</td>
</tr>
<tr>
<td>CSNV</td>
<td>Brazil</td>
<td>WUR</td>
</tr>
<tr>
<td>INSV</td>
<td>NL-07</td>
<td>WUR</td>
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<tr>
<td>MeSMV</td>
<td>Mexico</td>
<td>Istituto di Virologia Vegetale del CNR</td>
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<tr>
<td>BeNMV</td>
<td>Brazil</td>
<td>University of Brasília</td>
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<tr>
<td>IYSV</td>
<td>IYSV-NL</td>
<td>WUR</td>
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<tr>
<td>TYRV</td>
<td>Iran</td>
<td>WUR</td>
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<tr>
<td>PoIRSV</td>
<td>Plg3</td>
<td>Istituto di Virologia Vegetale del CNR</td>
</tr>
<tr>
<td>AYSV</td>
<td>ALS-2000</td>
<td>WUR</td>
</tr>
<tr>
<td>WSMoV</td>
<td>Thailand</td>
<td>WUR</td>
</tr>
<tr>
<td>GBNV</td>
<td>India</td>
<td>WUR</td>
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<tr>
<td>CaCV</td>
<td>Thailand</td>
<td>WUR</td>
</tr>
<tr>
<td>CCSV</td>
<td>Taiwan</td>
<td>National Chung Hsing University</td>
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<tr>
<td>TNRV</td>
<td>Thailand</td>
<td>WUR</td>
</tr>
<tr>
<td>MYSV</td>
<td>Thailand-physalis</td>
<td>WUR</td>
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<tr>
<td>PCFV</td>
<td>Taiwan</td>
<td>National Chung Hsing University</td>
</tr>
<tr>
<td>LNRV</td>
<td>Japan</td>
<td>Kochi Agricultural Research Center</td>
</tr>
</tbody>
</table>

Identity confirmed by sequencing of the entire N protein gene
Results testing different primer sets

Identification by amplicon sequencing
Conclusions

New generic and LNRV-specific primer sets allowed

• Detection of all included species

• Provisional identification by sequence analysis
## Results testing field samples

<table>
<thead>
<tr>
<th>Species</th>
<th>Crop species</th>
<th>Origin (number of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRSV</td>
<td><em>Capsicum annuum</em></td>
<td>Brazil (1)</td>
</tr>
<tr>
<td>INSV</td>
<td><em>Leontopodium sp.</em></td>
<td>Switzerland (1)</td>
</tr>
<tr>
<td>TCSV</td>
<td><em>Capsicum annuum</em></td>
<td>Brazil (1)</td>
</tr>
<tr>
<td>TCSV</td>
<td><em>Capsicum frutescens</em></td>
<td>Dominican Republic (1)</td>
</tr>
<tr>
<td>TSWV</td>
<td><em>Solanum lycopersicum</em></td>
<td>Croatia (1)</td>
</tr>
<tr>
<td>TSWV</td>
<td><em>Capsicum annuum</em></td>
<td>South Africa (1), Turkey (1)</td>
</tr>
<tr>
<td>CaCV</td>
<td><em>Capsicum annuum</em></td>
<td>China (1), Vietnam (1)</td>
</tr>
<tr>
<td>CaCV</td>
<td><em>Solanum lycopersicum</em></td>
<td>Vietnam (2)</td>
</tr>
<tr>
<td>GBNV</td>
<td><em>Solanum lycopersicum</em></td>
<td>India (3)</td>
</tr>
<tr>
<td>TNRV</td>
<td><em>Capsicum annuum</em></td>
<td>Thailand (6), Vietnam (1)</td>
</tr>
</tbody>
</table>
Conclusions and discussion

• Eurasian primer set might give background reaction in pepper (results not shown)

• Asian clade 1 primer set might react with IYSV (Eurasian clade)

• Amplicon sequences allow provisional identification

• American clade 1 and Asian clade 1 primers allow detection and identification of different species in various field samples

• Eurasian-primer set able to detect a ‘new’ tospovirus species (AYSV not used for primer design)

• Limited experience with American clade 2 and Asian clade 2 species so far (for both only one of two species tested)
Summary

• Five RT-PCR’s able to detect and provisionally identify the majority of known tospovirus species

• Fair chance to detect unknown species because of the generic primer design

• Important tool for diagnostic laboratories in plant health to prevent and/or control tospovirus infections
Acknowledgements

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