DIAGNOSIS OF CyMV AND ORSV ON Dendrobium AND Phalaenopsis THROUGH RT-PCR

Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) belong to the potexvirus and tobamovirus groups. They are two of the most prevalent and economically deleterious orchid viruses, which infect numerous commercially important orchid genera, and have attained a world-wide distribution. CyMV induces floral and foliar necrosis while ORSV causes ring spots on leaves and color breaking on flowers. Mixed infections of both viruses can cause blossom brown necrotic streaks. The viruses also reduce plant vigor and lower flower quality, thus affecting their economic value. This subject applied RT-PCR method combining with the detection by agarose gel electrophoresis and checked result by real-time PCR. The result showed that these methods provide highly sensitive and specific tests for orchid virus diseases.
CyMV and ORSV in orchid observed through microscope

*CyMV* and *ORSV* in orchid observed through microscope.
Primer design for PCR and Multiplex PCR

**IAS-POR1F**

371 bp

**IAS—POR1R**

**ORSV**

375 bp

**IAS-PCR1F**

306 bp

**IAS-PCM1R**

**CyMV**

742 bp

747 bp
Primer to detect ORSV
IAS-POR1F: 5’-TTG TTC GAT TAC TAC AAT TAC-3’ (21 nu)
IAS-POR1R: 5’-TAA TAG AAC CAA ATG GGA-3’ (18 nu)

Primer to detect CyMV:
IAS-PCM1F: 5’-TAA AGA CTT ATT GCG TTA CG-3’ (20 nu)
IAS-PCM1R: 5’-CAA CCA CTG CAG AGT GGA GT-3’ (20 nu)

FastPCR was used to check primers
Melt Curve exhibited cDNA by CyMV virus

RT-PCR products on agarose gel for ORSV and CyMV
Multiplex PCR products on agarose gel to monitor virus in meristem and PLB to develop healthy plants

Shoot samples

PLB samples (protocorm like body)
### Table: Percentage of infected plants by CyMV and ORSV

<table>
<thead>
<tr>
<th>Collected sample</th>
<th>Sample number</th>
<th>Infected sample (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CyMV</td>
</tr>
<tr>
<td>1 – Orchid farms</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>2 - <em>in vitro</em> orchids from laboratories</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>3 - <em>in vitro</em> orchids from seeds</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>4 - PLB regenerated from leaf tissues <em>in vitro</em></td>
<td>34</td>
<td>0</td>
</tr>
</tbody>
</table>
Hình 1: Mức độ huynh quang và số chu kỳ luân nhiệt

Hình 2: Biểu đồ Melt Curve

Hình 3: Mức độ huynh quang và số chu kỳ luân nhiệt

Hình 4: Biểu đồ Melt Curve