MOLECULAR DIAGNOSTICS FOR THE DETECTION OF STRAWBERRY VIRUSES

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INTRODUCTION

The supply of high-health, certified strawberry runners throughout Australia is dependent on the collections of nucleus plants maintained in Victoria and Queensland. Both collections are tested annually in spring using a biological indexing method of petiole grafting candidate tissue onto sensitive indicator species for several virus associated diseases. This method is reliable and sensitive only if done in spring or early summer. Biological indexing is also labour intensive, expensive and time consuming, taking 6-8 weeks to generate a result.

Recent advances in reverse-transcription polymerase chain reaction (RT-PCR) techniques have been published overseas for detection of Strawberry mothie sadwavirus (SMoV), Strawberry crinkle cytorhabdovirus (SCV), Strawberry mild yellow edge potexvirus (SMYEV), Strawberry vein banding caulimovirus (SBV), Beet pseudos yellows crinivirus (BPYV), and Strawberry pallidosis associated crinivirus (SPaV). These RT-PCR tests were adopted from international, peer-reviewed literature (2,3,4,5) and developed on positive control plants, maintained 12 months of the year in glasshouse conditions at DPI, Victoria. In 2006/07 field surveys were done in Victoria, Queens land and Western Australia to validate the molecular tests under local conditions. The results of the survey are reported below.

MATERIALS AND METHODS

Sampling. Sampling (whole plants or leaf and petiole samples) began in September 2006 and was completed in November 2006. A total of 99 samples were collected including commercial Fragaria sp (90/99) and Potentilla sp (6/99) and Fragaria X Potentilla hybrids (3/99).

Nucleic acid extraction, RT-PCR amplification and sequencing. Petioles were used for each RNA extraction. RT-PCR was done using an Invitrogen SuperScript™ III One-Step RT-PCR system with Platinum® Taq DNA Polymerase kit. PCR products were purified using the Qiagen QIAquick PCR purification kit (Qiagen) and cloned using the pGEM Teasy Vector system (Promega). The cloned products were sequenced using primers SP6 and T7, with an ABI PRISM® BIGDYE™ Terminator Cycle Sequencing Kit (App. Biosystems) and analysed using BlastN (1)

RESULTS and DISCUSSION

The number of samples that tested positive for each virus is listed in table 1. One or more viruses was detected in 23/119 samples.

The results indicate that strawberry viruses may not be widely distributed in Australia, indicating the success of the certification schemes in reducing the incidence of these pathogens. The low incidence of the viruses might be explained by re-infection in the field, once runners are released from the certification schemes.

Preliminary survey results indicated that the primer pair SMYEV/SMYEVdetb (3) were unreliable for SMYEV detection as they generated non-specific products similar in size to the expected product (false positives) in some samples. Sequencing indicated that this PCR product was associated with plant nuclear acid. Consequently, the primer pair SYEupStp1a/SYE PolyYb (2) was used for detection of SMYEV during the survey. This primer pair did not generate false positive results.

Two SPaV PCR tests were assessed. The CP5’/CP n731 (5) primer pair generated more positive results than the SP44F/SP44R (5) and were therefore more reliable for detection of SPaV. However, the SP44F/SP44R primer pair did detect one isolate SPaV that the former primer pair did not. Further work will be done to determine how genetic variation contributes to the reliability of the SPaV RT-PCR tests. A new test will be developed if required.

The PCR tests that have been developed and validated in this survey are rapid, cost effective and sensitive compared to biological indexing. During the 2007-2010 further research will be conducted to validate the tests on a "dummy" nucleus collection maintained under the same conditions as the Victorian nucleus collection. If required a field survey will be done to further validate the tests. The tests will support the strawberry runner certification schemes in Victoria and Queensland.

ACKNOWLEDGEMENTS

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REFERENCES


Table 1. The number of samples in which each strawberry virus was detected during the survey.

<table>
<thead>
<tr>
<th>Virus (primer name)</th>
<th>Positive results</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMoV</td>
<td>1/99</td>
</tr>
<tr>
<td>SMYEV</td>
<td>1/99</td>
</tr>
<tr>
<td>SCV</td>
<td>1/99</td>
</tr>
<tr>
<td>SPaV (CP5'/CP n731)</td>
<td>6/99</td>
</tr>
<tr>
<td>SPaV (SP44F/SP44R)</td>
<td>1/99</td>
</tr>
<tr>
<td>SBV</td>
<td>3/99</td>
</tr>
<tr>
<td>BPYV</td>
<td>0/99</td>
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</tbody>
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