Establishing a testing method for the aphid transmission of SPFMV and evaluating viral resistance in transgenic sweet potatoes

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The sweet potato, Ipomoea batatas (L.) Lam., is the third most important root crop in the world after the potato and cassava. In particular, the sweet potato is not only an important crop in tropical, subtropical and temperate regions, but also an efficient biomass-producing plant for starch. The sweet potato is widely recognised as being prone to infection by sweet potato feathery mottle virus (SPFMV). Generally, SPFMV is transmitted by aphids (Myzus persicae), and the infected tuberous roots are used for vegetative propagation. However, neither the transmission of SPFMV using the aphid transmission test nor the aphid transmit theory itself has been established for sweet potato. The present study establishes a testing method for the aphid transmission of SPFMV and evaluates viral resistance in transgenic sweet potatoes. As a result of some examinations, we establish a testing method for resistance to SPFMV by aphid transmission in sweet potato. Furthermore, we evaluated the resistance to SPFMV in transgenic sweet potato using the improved aphid transmission method, and determined higher levels of resistance to SPFMVs in transgenic sweet potatoes. These results suggest that transgenic sweet potatoes show resistance to SPFMVs in the field.

Key Words: aphid transmission, Myzus persicae, SPFMV resistance, transgenic sweet potato.

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inoculation feeding for 30 min. After the inoculation period, the aphids were killed by spraying an insecticide. Fourteen days after the inoculation, the plants were washed and the leaves, petioles and roots were sampled. The experiments were subsequently repeated three times (two plants each time). Viral detection was performed using RT-PCR, which was subject to the modification of Hanada et al. (1997). The total RNA was extracted from the samples (leaf, petiole and root) via the cetyltrimethylammonium bromide method and reverse transcription was performed using primers designed in the N1b-CP region of the Japanese isolates of SPFMV-O (Mori et al. 1994), S (Mori et al. 1995) and T (Sakai et al. 1995). A forward primer, TOS-1 (5′-GACAACACACTTAGTTGT-3′) and reverse primer, TOS-2 (5′-CGCGCAAGACTCATATCAGT-3′) were used to amplify a fragment of about 1.3 kb containing the CP region (Hanada et al. 1997). The total RNA was then reverse transcribed and amplified using the SuperScript™ One-Step RT-PCR kit (Invitrogen, Carlsbad, CA) following the manufacturer’s instructions. First strand-cDNA synthesis was conducted at 48°C for 20 min, followed by the thermal cycling scheme (35 cycles): initial denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, DNA synthesis at 68°C for 2 min and final extension at 72°C for 10 min. PCR products were separated by 1.5% agarose gel electrophoresis and visualised with ethidium bromide. Although no clear virus-derived signals were detected in any of the samples, a weak viral signal was detected from the root material sample (Table 1). Therefore, the root was used for further experiments.

Despite a few weak signals, the results of Experiments 1 confirmed that SPFMV was transmitted by M. persicae and that root was the optimal material for testing. In Experiment 2, we tried to optimize the testing procedure through improved starvation time, time of acquisition feeding, and cultivation method. Specifically, aperous aphids were starved for 4 hr and then allowed an acquisition feeding for 10 sec on leaves of infected sweet potato. The inoculation feeding time of 30 min remained unchanged, while the cultivation method was changed to aquaculture. In addition, the cultivation time was extended to one month to allow an increase in the viral load. Viral infection was again tested by RT-PCR, while RNA extraction, primer design and RT-PCR were performed as described above. The experiments were repeated three times (with two plants each time). Consequently, a clear virus-derived signal was detected in the root material (Table 1 and Fig. 1). This improved method should therefore be suitable to test for aphid transmission. In contrast, viral detection using leaf material was inconsistent (Fig. 1). In our previous study (Okada and Saito 2008), the viral concentration and detection of combinations of virus strains by RT-PCR and the Western blot method differed between leaves in the same stock. The same variability was seen in the viral detection of leaves using the aphid transmission test. Root material from plants cultivated in aquaculture was therefore thought to be most effective for these transmission tests. Figure 2 summarizes the modified aphid transmission method used in this study. The results showed the establishment of a testing method for resistance to SPFMV by aphid transmission in sweet potato.

Finally, we evaluated the resistance to the virus in transgenic sweet potato using the improved aphid transmission method established in Experiment 2. In this study, the defined virus resistance was not detected in root materials using the established aphid transmission method. All the

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**Table 1. Aphid transmission of SPFMV from infected sweet potato (Ipomoea batatas) to healthy I. batatas using Myzus persicaea**

<table>
<thead>
<tr>
<th>Aphid transmission test</th>
<th>Time of starvation</th>
<th>Time of acquisition feeding</th>
<th>Time of inoculation feeding</th>
<th>Cultivation method</th>
<th>Cultivation period</th>
<th>No. of RT-PCR detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>General aphid transmission (Experiment 1)</td>
<td>2.0 hr</td>
<td>10 min</td>
<td>30 min</td>
<td>Soil culture</td>
<td>14 days</td>
<td>0/30</td>
</tr>
<tr>
<td>Modified aphid transmission (Experiment 2)</td>
<td>4.0 hr</td>
<td>10 sec</td>
<td>30 min</td>
<td>Water culture</td>
<td>30 days</td>
<td>5/12</td>
</tr>
</tbody>
</table>

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"a" Ten aphids per one plant (I. batatas) were used in each test.

"b" Number of samples that were weakly detected by RT-PCR. No clear signals were obtained.

n.t. Not tested.

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Fig. 1. Testing via inoculation of SPFMV by aphid (Myzus persicae) transmission. M. 2-Hind III; 1, virus infected plant; 2, virus free plant; 3–4, aphid-inoculated cv. Chikei 682-11(1) (leaf); 5, aphid-inoculated cv. Chikei 682-11(1) (root); 6–7, aphid-inoculated cv. Chikei 682-11(2) (leaf); 8, aphid-inoculated cv. Chikei 682-11(2) (root); 9–10, aphid-inoculated cv. Kokei No. 14 (leaf); 11, aphid-inoculated cv. Kokei No. 14 (root).
experimental conditions were set up as described in Experiment 2 and the experiments were repeated five times (with two plants each time). One month after the inoculation, the viral infection and the extent of its spread were assessed by RT-PCR. All transgenic sweet potato plants increased resistance to SPFMVs as compared with control plants (Table 2 and Fig. 3). Previously, we investigated resistance to SPFMVs by graft-inoculation on the same transformant, and found the plants to be highly protected against SPFMVs (Okada and Saito 2008). Graft-inoculation represents the most potent, high dosage, continuous supply of virus inoculum to a plant (Wroth and Jones 1992, Njeru et al. 1995). On the other hand, SPFMV is transmitted by aphids under natural conditions. In this study, higher levels of resistance to SPFMVs in transgenic sweet potatoes were found when the method of inoculation was by aphid transmission. These results suggest that transgenic sweet potatoes show resistance to field SPFMVs. Further field experiments are now being planned.

Acknowledgments

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<table>
<thead>
<tr>
<th>Aphid transmission test</th>
<th>Virus free</th>
<th>Virus detected roots / Investigated roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified aphid transmission (Experiment 2)</td>
<td>—</td>
<td>9/10 — 10/10 10/10 0/10 0/10 0/10</td>
</tr>
</tbody>
</table>

* Ten aphids per one plant (*I. batatas*) were used in each test.
— not detected.

**Table 2.** Evaluation of resistance to SPFMV in transgenic sweet potatoes infected by aphid transmission using RT-PCR.

**Fig. 2.** Procedure for the modified aphid transmission method for the inoculation of SPFMV.

**Fig. 3.** Evaluation of transgenic sweet potato resistance to SPFMVs by the modified aphid transmission method. M, λ-Hind III; 1, virus infected plant (leaf); 2, virus infected plant (root); 3, virus free plant (root); 4–5, aphid-inoculated non-transformant (root); 6–7, aphid-inoculated transgenic plant EP200-1 (root); 8–9, aphid-inoculated transgenic plant EP200-2 (root); 10–11, aphid-inoculated transgenic plant EP220-1 (root); 12–13, aphid-inoculated transgenic plant EP220-2 (root).

**Literature Cited**


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