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Identification of Potato Y Potyvirus (PVY\textsuperscript{O}) Resistance in Wild and Cultivated Tomatoes

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Abstract: Potato Y potyvirus (PVY) is an important plant pathogen worldwide that infects and causes yield losses in the family Solanaceae including potato (Solanum tuberosum), pepper (Capsicum spp.), tomato (S. lycopersicum), and tobacco (Nicotiana tabacum). In this study, 20 different tomato accessions representing 6 different species were mechanically inoculated with PVY\textsuperscript{O}. The plants were scored visually for symptoms and then tested for presence of the virus 2-4 weeks after inoculation by ELISA. The results were variable. Most wild species of tomato sustained PVY\textsuperscript{O} replication in inoculated leaves. Some of the wild species showed an immune response, while some became systemically infected. Inoculation and analysis of F\textsubscript{2} populations suggested that the resistance is controlled by a single recessive gene in different wild species.

Key Words: Immunity, resistance, PVY\textsuperscript{O}, tomato

Introduction

Tomato is one of the world's most important vegetable crops and various diseases limit optimal production in terms of both quality and quantity (yield) (Sikora et al., 1998). Potato virus Y (PVY) is a widely studied type member of the genus Potyvirus (De Bokx and Huttinga, 1981; Spetz et al., 2003). PVY is one of the most agriculturally important viruses infecting plants in the family Solanaceae, which includes tomato (Solanum lycopersicum), potato (S. tuberosum), tobacco (Nicotiana tabacum), eggplant (S. melongena), and pepper (Capsicum spp.) (Boonham and Barker, 1998; Jeffries, 1998; Stevenson et al., 2001; Glais et al., 2002).
There are many different strains of PVY. In nature, 3 groups of PVY strains are potato strains and they have been identified by symptoms. PVY\textsuperscript{O} is the ordinary or common strain and causes mild to severe mosaic symptoms on potato. This strain was first identified by Smith (1931). PVY\textsuperscript{N}, the necrotic strain, was recognized in the 1950s and causes severe vein necrosis (Singh, 1992). PVY\textsuperscript{C}, the stipple streak strain, causes stipple streak in tubers. It was first described by Beczner et al. (1984).

PVY is transmitted in a nonpersistent manner by many aphid species (Varveri, 2000). Aphids can acquire the virus in less than 60 s from an infected plant and transmit it to a healthy plant in less than 60 s. PVY can also be transmitted mechanically (Whitham and Wang, 2004).

The resistance mechanism of PVY has been the subject of several studies. Resistance genes that control PVY have been identified in cultivated and wild potato species and have been used in potato breeding for many years (Cockerham, 1970; Jones, 1990). Extreme resistance genes, \( R_{Y_dig} \) and \( R_{Y_{dig}} \), that confer complete resistance to infection at the whole plant level (immunity) have been mapped on chromosome XI in potato by Brigneti et al. (1997) and Hamalainen et al. (1997), respectively. Another study by Valkonen et al. (1994) showed that \( S. tuberosum andigena \) has 2 resistance genes, namely \( R_{Y_{dig}} \), controlling extreme resistance to PVY\textsuperscript{O}, and \( N_{Y_{dig}} \), controlling hypersensitive resistance to PVY\textsuperscript{O}. In addition, the extreme resistance gene is effective against all strains of PVY (Cockerham, 1970; Jones, 1990; Volkanen et al., 1994). However, the hypersensitive resistance gene is often strain group-specific (Hamalainen et al., 1997; Volkanen et al., 1994). Potato plants that carry the gene(s) for a hypersensitive response to the virus develop some necrosis after inoculation. This necrosis may be limited to inoculated tissue or may spread throughout the plant and result in death (Bawden, 1936; de Boekx and Huttinga, 1981; Jones, 1990; Valkonen, 1994). A limited necrotic response may lead to resistance to the virus, while a systemic response will cause total crop loss. The hypersensitive response gene was mapped on chromosome IV of potato (Celebi-Toprak et al., 2002). However, this resistance mechanism has not been well studied in tomato. There are only a limited numbers of studies on this subject. The wild species of tomato harbor genes for resistance and tolerance to a wide range of diseases and insects (Banerjee and Kalloo, 1987; Kalloo and Banerjee, 1990; Giordano, 2005). Specifically, \( S. habrochaites \) lines (e.g. PI 247087) are known sources of resistance to PVY strains (Thomas, 1981; Gebre-Selassie et al., 1987; Legnani et al., 1995). The recessive resistance gene, \textit{pot-1}, was mapped on tomato chromosome III and provides resistance to 2 potyviruses (Parrella et al., 2002). Despite this knowledge, to date no PVY resistant cultivar of tomato has been developed (http://www.avrdc.org/pdf/tomato/PVY.pdf). Therefore, it is very difficult to control the virus, and once a crop is infected there is no effective way to limit losses. Resistant varieties represent the simplest, safest, and most effective strategy to limit losses to this pathogen in tomato.

Tomato is a well-developed model system for molecular genetic studies. Thus, once PVY resistance sources are identified, molecular markers can be used to facilitate the localization and transfer of PVY resistance gene(s) from unadapted wild germplasm to elite cultivated types. The objective of this study was to screen wild species and cultivated tomato to identify resistance to PVY\textsuperscript{O}.

Materials and Methods

\section*{Plant materials and populations}

A total of 253 plants from 20 different tomato accessions (6 different species) were mechanically inoculated to test for their susceptibility to PVY\textsuperscript{O} infection: 2 accessions of \( S. lycopersicum \) (syn Lycopersicon esculentum), 7 accessions from \( S. chilense \) (syn \( L. chilense \)), 1 accession of \( S. chmielewskii \) (syn \( L. chmielewskii \)), 3 accessions from \( S. cornelio-muelleri \) (syn \( L. glandulosum \)), 3 accessions from \( S. habrochaites \) (syn \( L. hirsutum \)), and 4 accessions of \( S. peruvianum \) (syn \( L. peruvianum \)). Accession numbers and sources of these lines are given in Table 1. In addition, selected F\textsubscript{1} hybrids and F\textsubscript{2} populations were tested with PVY\textsuperscript{O}. Two interspecific hybrids were tested: \( S. habrochaites \) LA1223 \( \times \) \( S. lycopersicum \) cv. E6203 and \( S. chmielewskii \) PI379030 \( \times \) \( S. lycopersicum \) cv. E6203. Three \( S. habrochaites \) F\textsubscript{2} populations and 1 \( S. chmielewskii \) F\textsubscript{2} population derived from the F\textsubscript{1} hybrids were also inoculated with PVY\textsuperscript{O}. All F\textsubscript{1} and F\textsubscript{2} seeds were obtained from İzmir Institute of Technology, Urla, İzmir, Turkey. Plants were maintained in the greenhouse at approximately 22 to 25 °C with a 16-h photoperiod.
Seeds were germinated in 2 × 2 cm 180-well seedling trays in a climate-controlled greenhouse. They were transferred into 10-cm pots 2 weeks after germination. Potato plants (S. tuberosum) were used as inoculum sources and as positive controls.

In this study, plant responses are classified as follows: 1) Extreme resistance (immune): the plants were able to inhibit virus infection not only systemically but also at the points of inoculation, in other words, virus replication was not detected by ELISA and plants did not show any symptoms. 2) Resistance: PVY replication in inoculated leaves but not in new developing leaves was detected by ELISA, and the plants did not show any symptoms. 3) Susceptible: PVY replication both in inoculated and new developing leaves was detected by ELISA and the plants showed symptoms.

Viral isolate

The PVY isolate used in this study belongs to the strain group (PVY\(O\)) and was originally isolated from the S. tuberosum clone MexSS 1035 (PI383471). The isolate was propagated and maintained in susceptible potatoes. Relative virus concentrations in plants were monitored by the enzyme linked immunosorbent assay (ELISA) to assure high inoculum titer in tissue used for inocula.

Mechanical inoculation

Young tomato plants were inoculated when they had approximately 3-4 leaves. Mechanical inoculation was performed according to Celebi-Toprak et al. (2002). Two plants of each genotype and 2 tobacco plants were inoculated with phosphate buffer as negative controls. Two tobacco plants and susceptible potato cultivars were used as positive controls for inoculation.

ELISA

Samples consisting of (1) inoculated leaves and (2) 2 leaves positioned 2 nodes above the inoculated leaves representing new growth and apical leaves were taken per plant, 2 and 4 weeks after inoculation, respectively. Samples were scored visually for symptoms and then tested by a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977). Monoclonal alkaline peroxidase phosphatase-conjugated antibody to PVY\(O\) was obtained from Agdia (Elkhart, Ind.).

Data analysis

Chi square goodness-of-fit tests were used for genetic analysis.

Results

ELISA results from S. lycopersicum tomato accessions

Two different lines from S. lycopersicum were tested. These accessions were used as positive controls. The results are shown for each accession (Table 1). All plants that were tested from S. lycopersicum LA1995 and S. lycopersicum cv. E6203 were susceptible. PVY\(O\) replication in susceptible plants was detected in both inoculated and new developing leaves by ELISA. Furthermore, systemic spread of PVY\(O\) in S. lycopersicum was expressed with typical symptoms: mosaic, leaf deformation, a droopy appearance with curved petioles and leaves rolled downward.

ELISA results from S. chilense tomato accessions

Seven different accessions from the wild-type tomato S. chilense were tested. All tested tomato lines were immune. The virus did not replicate in inoculated leaves and systemic virus spread did not occur in S. chilense accessions LA 1963, 1930, 1938, 1971, 1932, 1960, or 1958. These results showed that these plants are immune to PVY\(O\) (Table 1).

ELISA results from S. chmielewskii tomato accessions

Fifteen plants were tested from S. chmielewskii PI 379030: 7 plants were immune and 8 plants were susceptible to PVY\(O\) (Table 1). The virus was not detected by ELISA in either inoculated or new developing leaves. Furthermore, these plants did not show any symptoms.

ELISA results from S. corneliomuelleri tomato accessions

Three different accessions from S. corneliomuelleri were tested and each gave different results (Table 1). Susceptible plants had virus replication in both inoculated and new developing leaves and showed symptoms. The resistant plants of the 3 S. corneliomuelleri lines showed 2 different types of response. For 3 (43%) of the S. corneliomuelleri CGN 14357 resistant plants and 4 (29%) of the resistant PI 126443 plants, the virus replicated in inoculated leaves but not in new developing leaves. Virus replication was detected in inoculated leaves by ELISA. For the remaining resistant plants of these lines (57% and 71%, respectively) and all of the CGN 15802 resistant plants, the virus did not replicate in inoculated or new developing leaves. These results indicated that these
plants were immune to PVY\textsuperscript{O}. Neither resistant nor immune plants showed any symptoms.

**ELISA results from *S. habrochaites* tomato accessions**

The results from 3 different accessions of wild type *S. habrochaites* appeared to show resistance to PVY\textsuperscript{O} (Table 1). All plants from *S. habrochaites* accession PI 247087 showed immunity to PVY\textsuperscript{O}, as the plants were able to inhibit virus infection not only systemically but also at the points of inoculation and plants did not show any symptoms. In addition, plants from *S. habrochaites* LA 1223 and LA 1777 showed resistance to PVY\textsuperscript{O}, but in this case the virus replicated in inoculated leaves but not in new developing leaves. Some plants from *S. habrochaites* LA 1223 and LA 1777 showed susceptible responses to PVY\textsuperscript{O}. PVY\textsuperscript{O} replication both in inoculated and new developing leaves was detected by ELISA and the plants showed symptoms.

**ELISA results from *S. peruvianum* tomato accessions**

Four different accessions from *S. peruvianum* were tested. The results were variable (Table 1). Susceptible plants had virus replication in both inoculated and new developing leaves, and showed symptoms. PVY\textsuperscript{O} was detected by ELISA. The resistant plants of the 4 *S. peruvianum* lines showed 2 different types of response. For all resistant plants of PI 128654 and 1 plant (17\%) of PI 128657, the virus replicated in inoculated leaves but not in new developing leaves, and no symptoms were observed. For the remaining resistant plants of the line PI 128657 (83\%) and all of the PI 126444 and PI 128660 resistant plants, the virus did not replicate in inoculated or

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession (source)</th>
<th>Total number of plants inoculated</th>
<th>Susceptible plants number</th>
<th>Total number of resistant plants</th>
<th>Immune plants number</th>
<th>Resistant plants number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. lycopersicum</em></td>
<td>LA 1995 (3)</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>cv. E6203 (4)</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><em>S. chilense</em></td>
<td>LA 1963 (3)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LA 1930 (3)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LA 1938 (3)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LA 1971 (3)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LA 1932 (3)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LA 1960 (3)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LA 1958 (3)</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
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<tr>
<td><em>S. chmielewskii</em></td>
<td>PI 379030 (2)</td>
<td>15</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>0</td>
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<tr>
<td><em>S. cornelioniuelleri</em></td>
<td>CGN 14357 (1)</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>CGN 15802 (1)</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PI 126443 (2)</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>10</td>
<td>4</td>
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<tr>
<td><em>S. habrochaites</em></td>
<td>LA 1223 (3)</td>
<td>32</td>
<td>20</td>
<td>12</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>LA 1777 (3)</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>PI 247087 (2)</td>
<td>23</td>
<td>0</td>
<td>23</td>
<td>23</td>
<td>0</td>
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<tr>
<td><em>S. peruvianum</em></td>
<td>PI 128657 (2)</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>1</td>
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<tr>
<td></td>
<td>PI 126444 (2)</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PI 128660 (2)</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PI 128654 (2)</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

\textsuperscript{2}Sources are coded: 1 = Genetic Resources Centre, Netherlands; 2 = United States Department of Agriculture Research Station, New York, USA; 3 = Tomato Genetics Resource Center, Davis, CA, USA; 4 = İzmir Institute of Technology, İzmir, Turkey. Pl. I.: plant inoculated. Sus. Pl: susceptible plants. Im Pl.: immune plants. Res. Pl.: resistant plants.
new developing leaves. No symptoms were observed in these lines. These results indicated that these plants were immune to PVYO.

ELISA results from *S. habrochaites* F₁ hybrids and F₂ populations

*S. habrochaites* F₁ hybrids were generated from a cross between *S. habrochaites* LA1223 and *S. lycopersicum* cv. E6203. Thirteen hybrid (F₁) plants were tested. Seven of the hybrids were resistant while 6 were susceptible. All of the resistant plants were classified as immune. Three different F₂ populations were generated from the F₁ hybrids and were tested with PVYO. All of the resistant plants from the 3 different lines of *S. habrochaites* showed an immune response. PVYO did not replicate in inoculated leaves and new developing leaves, and no symptoms were observed in immune plants. However, for susceptible plants of the 3 different F₂ populations, PVYO replicated in inoculated and new developing leaves, and symptoms were observed.

When segregation data from the F₂ populations were analyzed they were consistent with a 1R:3S ratio (P = 0.643, P = 0.506, P = 0.87), expected under the hypothesis that the gene controlling resistance to PVYO has monogenic recessive inheritance (Table 2).

ELISA results from *S. chmielewskii* F₁ hybrids and F₂ populations

Thirteen hybrids from *S. chmielewskii* F₁ (cv. E6203 × PI379030) were tested with PVYO. Three of the hybrids were resistant while 10 were susceptible. A *S. chmielewskii* F₂ population was generated from the F₁ and 70 plants from this population were tested. The immune plants did not show any symptoms. Moreover, the virus was not detected in immune plants by ELISA. However, in susceptible plants, the virus was detected in both inoculated and new developing leaves by ELISA, and symptoms were observed.

When segregation data from the F₂ population were analyzed, results from the F₂ populations were consistent with a 1R:3S ratio (P = 0.214), expected under the hypothesis that the gene controlling resistance to PVYO is monogenic and inherited recessively (Table 2).

Discussion

A total of 253 plants from 20 different tomato accessions (6 different species) were mechanically inoculated with PVYO. The plants were tested 2-4 weeks after inoculation by ELISA. The results were variable. Most wild species of tomato sustained PVY replication in inoculated leaves. Some of the wild species showed an immune response, while some became systemically infected.

The results from 3 different accessions of wild type *S. habrochaites* appeared to show resistance to PVYO (Table 1). Similar results were found in previous studies by Thomas (1981), Thomas and MacGrath (1988), Gebre-Selassie et al. (1987), and Legnani et al. (1995). They showed that *S. habrochaites* PI 247087 was immune to PVYO and that the resistance is controlled by a recessive gene. In this study, all plants from this accession showed immunity to PVYO, as the plants were able to inhibit virus infection not only systemically but also at the points of inoculation. Furthermore, the resistant plants neither developed visible symptoms nor contained detectable levels of virus according to ELISA. Brigneti et al. (1997) suggested that resistance affected virus replication or virus stability. Thus, the extreme resistance gene from the resistant wild tomato species may act by inhibiting PVY replication or negatively impacting virus stability. Legnani et al. (1995) also showed that the resistance of *S. habrochaites* PI 247087 is effective against various isolates or strains. Inoculation with high concentrations of purified PVY could not overcome this resistance.

<table>
<thead>
<tr>
<th>F₂ population</th>
<th>Resistant plant number (immunity)</th>
<th>Susceptible plant number</th>
<th>Expected ratio 1R:3S</th>
<th>χ² value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. habrochaites</em> F₂ 1518-4</td>
<td>11</td>
<td>28</td>
<td>1:3</td>
<td>0.214</td>
<td>0.643</td>
</tr>
<tr>
<td><em>S. habrochaites</em> F₂ 1518-9</td>
<td>11</td>
<td>26</td>
<td>1:3</td>
<td>0.441</td>
<td>0.506</td>
</tr>
<tr>
<td><em>S. habrochaites</em> F₂ 1518-16</td>
<td>26</td>
<td>81</td>
<td>1:3</td>
<td>0.028</td>
<td>0.87</td>
</tr>
<tr>
<td><em>S. chmielewskii</em> F₂</td>
<td>13</td>
<td>57</td>
<td>1:3</td>
<td>0.342</td>
<td>0.214</td>
</tr>
</tbody>
</table>
Temperature also did not affect the expression of the resistance of PI 247087 to PVY (Legnani et al., 1995). This resistance gene is effective against all strains of PVY and was mapped in the tomato genome (Parrella et al., 2002).

The results showed that extreme resistance (immunity) and resistance to PVYO were present in most wild tomato species. To our knowledge, this is the first time that extreme resistance (immunity) to PVY has been reported in S. chilense, S. chmielewskii, S. corneliomuelleri, and S. peruvianum.

The immune resistance responses observed in S. habrochaites and S. chmielewskii F1 populations appear to be controlled by single recessive genes. Further analysis will be needed to determine if the resistance in these 2 species is due to the same allele, different alleles of the same locus, or different loci. Once the PVYO resistant locus/loci are identified, it will then be possible to transfer them from unadapted wild germplasm to commercially acceptable tomato cultivars by breeding and marker-assisted selection.

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