DETECTION OF PVY (POTATO Y POTYVIRUS), ON POTATO CULTIVARS USING BIOLOGICAL AND MOLECULAR METHODS GROWING IN SOUTH-WEST TURKEY

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ABSTRACT

A large number of pathogens, cause economically important diseases on potato plants worldwide. Among these pathogens, potato virus Y potyvirus is one of the most common pathogens of potato. In this study, observations of mosaic patterns on leaves, stunting of the plant, leaf malformations, on potato plants in this region during field surveys indicated the presence of PVY in South-West region in Turkey. To test the presence of this virus in potato fields, leaf samples were collected from different areas in the region. In total 54 (52 potato and 2 N. glutinosa) samples were first tested by reverse transcriptase-polymerase chain reaction (RT-PCR) assay using primers specific to coat protein gene of PVY. As a result of RT-PCR, 23 potato leaf samples (42.33%) were determined as positive. RT-PCR positive some of the samples were then used in biological studies.

Keywords: PVY; Potato; RT-PCR; Molecular characterization.

INTRODUCTION

Potato is affected by many pests and numerous fungal, bacterial and viral diseases. Among these diseases, viruses cause in the field and during post-harvest varies from mild symptoms to heavy crop losses. A large number of RNA viruses cause economically important diseases on potato plants worldwide. Most of these viruses are biologically and taxonomically different and belong to different genera of single strand positive sense RNA viruses. The most common RNA viruses found in potatoes are Potato virus Y (PVY), Potato Virus X (PVX), Potato leafroll virus (PLRV) Potato Virus A (PVA), Potato viruses S and M (PVS and PVM) (Burrows and Zitter, 2005). These viruses are widely distributed in potato production areas of the world. Potato virus Y, is one of the most common pathogens of potato worldwide (Salazar, 2003, Brunt, 2001, Van Regenmortel et al., 2000, Rigotti and Gugerli, 2007). It is characterized by a broad range of hosts that include both monocotyledonous and dicotyledonous plants. PVY is among the five most economically damaging viruses (Rolland et al., 2008), with a host range including major crops, such as pepper (Capsicum annum L.), potato (Solanum tuberosum L.), tobacco (Nicotiana spp), tomato (Lycopersicon esculentum Mill.), less important plants and several species of weed mainly in the Solanaceae family (Karlan and Moury, 2008).

One factor that contributes to PVY’s importance is that it is readily transmitted by several species of aphids, common pests of potatoes. It is transmitted in a non-persistent manner by several aphid species. M. persicae is the most efficient and common vector in nature. PVY is extremely variable and three groups of strains are recognized (PVYO, PVYN and PVYC). The most damaging one at present is PVYN that causes ringspots on the tubers. Therefore virus can cause heavy yield losses and are a serious threat to potato production (Valkonen, 2007, Karasev and Gray, 2013).

In Turkey, virus infections tend to increase day by day in the country due to uncontrolled sales and certification of seeds and ineffective control of virus vectors. Commercial production of potato is primarily through vegetative propagation by means of tubers. For this reason, many viruses are transmitted from generation to generation and region by means of infested tubers (Burrows and Zitter, 2005).

PVY is a member of the family Potyviridae with non-enveloped filamentous, flexuous rod virions. PVY has positive-sense single-stranded linear RNA genome (Shukla et al., 1994). In potato it causes, mosaic or rugose mosaic, mild to severe mottle distortion (crinkling) of the leaves, vein necrosis and necrotic spots (Karlan, 2006). Symptoms are variable depending on viral strain, host cultivar, climatic conditions, and whether it is a primary infection (inoculation by aphid vectors) or secondary infection (when mother tuber is infected) (Draper et al., 2002).

Even though the region is an important potato production area, viral diseases in fields in this region are largely unknown because of insignificant attention given to viral diseases in the region.

In this study, PVY was detected and identified in potato growing in the Southwest region using different methods including biological indexing and RT-PCR. Some potato viruses has previously been detected in different region of Turkey (Arlı-Sökmen et al., 2005,
Materials and Methods

Field Surveys: Surveys were conducted in the springs of 2011 to 2012 in commercial potato growing areas of Isparta province located in Southwest Turkey. A total of 52 virus bearing suspected leaf samples were collected from potato plants (Solea, Safran, Floris, Proventa, Milva, Universa, Vangogh, Marabel). Symptoms of plants were recorded before putting leaf samples into plastic bags and storing in a freezer at -20°C until RT-PCR tests were done. During surveys, virus suspected plants were photographed in the fields.

Total RNA Extraction: Total RNA was extracted from fresh leaves of potato samples. H2O was used as a negative control. In RT-PCR study, a sample yielding positive results in previous ELISA test was used as positive control (Yardmcı et al., 2013). EZ-10 spin column plant total RNA minipreps kit (Bio Basic, Canada Inc) was used to extract the total RNA from infected plants. The extraction was done by following the procedure described by the manufacturer.

RT-PCR: The reverse transcription and polymerase chain reaction amplification was performed using OneStep RT-PCR Kit (Bio Basic, Canada Inc). Reverse transcription was performed in a 50 l reaction mixture containing, 21 l H2O, 25 l 2x1 PrimeScript One Step RT-PCR buffer (containing dNTP mixture, One step Enhancer solution), 2 l Prime Script 1 step enzyme mix, 1 l 20 Mm primers. Oligonucleotide primer sequences reported by Shalaby et al., (2002) were used to detect PVY.

Primer I: 5’TCAAGGATCCGCAAATGACACAATGGATGCAGG 3’
Primer II: 5’AGAGAGAATTTCATCACATGGTTCTGACTCC 3’

The amplified fragment was in length of 801 bp. The primer set were synthesized by Bio Basic, Canada Inc. Thermocycling was carried out as follows: 50°C for 30 min., 94°C for 2 min., then 30 cycles of 94°C for 30 second, 55°C for 30 second and 72°C for 1 min., followed by 72°C for 3 min. PCR products were separated in 1% agarose gel by electrophoresis, stained with 0.5 g/ml ethidium bromide solution. An image was captured after exposing the ethidium-bromide-stained gel on a transilluminator with a digital camera (UVP-Doc-It). DNA markers (100 bp DNA ladder, Fermentas; 1 kb DNA ladder, Takara) were used in each electrophoretic run.

Results and Discussion

Field Survey Studies: Surveys were conducted in potato growing areas of Isparta Province located in Southwest Turkey. A total of 52 leaf samples were collected from potato plants. Number of samples collected from these areas are shown in Table 1. During field surveys virus-like symptoms including mosaic patterns on leaves, stunting of the plant, leaf malformations were observed in potato plants. Some of these symptoms photographed during field studies are presented in Figure 1 (A,B,C). Observation of the presence of these symptoms indicated that potato plants in the visited fields were possibly infected with some viruses. Similar observations were reported in different studies (Stevenson et al., 2001, Kerlan, 2006, Kogovsek et al., 2011).

RT-PCR Studies: In order to determine the presence of PVY in samples, RT-PCR studies were carried out. As a result of RT-PCR, when using RT-PCR studies, in 23 potato leaf samples (44.23 %) and showing systemic infections N. glutinosa samples bands of expected approximate size for PVY 801 bp were observed (Figure 2. A,B,C,D,E,F). Similar results were reported in different studies (Shalaby et al., 2002, Amer et al., 2004, Zhiming et al., 2005, El-Araby et al., 2009, Gawande et al., 2011, Al-Saikhan et al., 2014).

In this study, RT-PCR which is sensitive and specific technique for rapid detection and amplification of PVY coat protein gene using a specific primer for coat protein, was utilized successfully to detect PVY in infected plant materials. RT-PCR positive some of the samples were then used in biological studies.

Biological Assay Studies: RT-PCR positive isolates were mechanically inoculated onto Chenopodium amaranthicolor, C. quinoa, Nicotiana glutinosa, N. tabacum White Burley and N. tabacum Xanthii plants. Inoculated plants were maintained in a greenhouse under natural light and ambient temperature.

After mechanical inoculations, systemic mosaic, wrinkle, leaf deformations and stunting were observed on N. glutinosa plants (Figure 3). Symptom development was observed after two weeks inoculation. Inoculated
other test plants did not show any symptoms. Similar results were in different studies (Crescenzi et al., 2005; Lorenzen et al., 2006; Kogovsec et al., 2011). The PVY was isolated from N. glutinosa leaves with systemic symptoms and used RT-PCR studies.

Biological, serological and molecular assays have generally been used for identification of PVY (Gawande et al., 2011, Mallik et al., 2012, Singh et al., 2013). Although ELISA is preferred assay for routine virus detection, RT-PCR has increasingly been used for detection and identification of viruses due to higher level of sensitivity. Therefore we used RT-PCR assays for detection of PVY in this study. In the study we found that PVY was present in this region of potato production areas by biological and RT-PCR methods.

Various viruses have previously been detected in the other regions of the country, however this is the first time that PVY was determined by RT-PCR methods in this region of Turkey. In the surveys conducted on potato viruses in Turkey, PVX and PVY were found (Bostan and Haliloğlu, 2004, Güner and Yorgancı, 2006).

In this study, using potato varieties of leaf samples, it was found that a high ratio of potato samples were infected with PVY (44.23 %). The occurrence and wide distribution of PVY in potato plants were most likely related to the large abundance of aphids in this region. Fourteen aphid species belonging to eight genera and three families of the superfamily Aphidoidea were present in the Isparta region (Aslan and Karaca, 2005). Thus, control of vectors is one of the most important methods for controlling PVY and other potato viruses in this region. Besides, in consequence of the infestation of all potato varieties in the region with the viruses, use of certified seed potato tubers and resistant varieties are necessary for the virus-free potato production.

As a result of RT-PCR, 23 potato leaf samples (44.23 %) were determined as positive and in these samples bands of expected approximate size for PVY 801 bp were observed. Showing systemic infections N. glutinosa plants were also used in RT-PCR studies. When using RT-PCR studies N. glutinosa samples bands of expected approximate size for PVY 801 bp were observed in RT-PCR detection.

![Figure 1. A) Mosaic patterns and interveinal yellowing on potato leaves. B) Leaf necrosis symptoms. C) Mottling, streaking and mosaic on leaves.](image-url)
Figure 2. A.B.C.D.E.F. Agarose gel electrophoretic stained by ethidium bromide analysis of RT-PCR amplification of PVY cp gene from infected field samples of potato. M\textsubscript{1}: Marker 100 bp DNA ladder, M\textsubscript{2}: Marker 1 kb DNA ladder, P: Positive control, N: Negative control, Lanes: infected field samples.

Figure 3. Systemic symptoms were observed on inoculated leaves *N. glutinosa*. 
Table 1. Varieties of potatoes, number of collecting samples and number of samples infected with PVY and infection ratio of PVY in samples determined by RT-PCR.

<table>
<thead>
<tr>
<th>Name of variety</th>
<th>Collected samples</th>
<th>Infected samples</th>
<th>Disease ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universa</td>
<td>13</td>
<td>7</td>
<td>53.84</td>
</tr>
<tr>
<td>Marabel</td>
<td>6</td>
<td>4</td>
<td>66.66</td>
</tr>
<tr>
<td>Milva</td>
<td>5</td>
<td>2</td>
<td>40.00</td>
</tr>
<tr>
<td>Solea</td>
<td>10</td>
<td>5</td>
<td>50.00</td>
</tr>
<tr>
<td>Vangogh</td>
<td>3</td>
<td>1</td>
<td>33.33</td>
</tr>
<tr>
<td>Floris</td>
<td>5</td>
<td>2</td>
<td>40.00</td>
</tr>
<tr>
<td>Safran</td>
<td>5</td>
<td>1</td>
<td>20.00</td>
</tr>
<tr>
<td>Provena</td>
<td>5</td>
<td>1</td>
<td>20.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>52</strong></td>
<td><strong>23</strong></td>
<td><strong>44.23</strong></td>
</tr>
</tbody>
</table>

**REFERENCES**


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