Detection of PVY, PVX, PVS, PVA, and PLRV on Different Potato Varieties in Turkey Using DAS-ELISA

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ABSTRACT

This research was performed in order to study and diagnose Potato virus Y (PVY), Potato virus X (PVX), Potato virus S (PVS), Potato virus A (PVA) and Potato leafroll Luteovirus (PLRV) on tubers and leaves of different potato varieties, namely, Solea, Safran, Floris, Proventa, Milva, Universa, Lady olympia, Vangogh, and Marabel grown in Afyon region of Turkey. For this purpose, potato tubers from different varieties were obtained from Afyon region producers during 2009-2010 and they were planted in the trial plots in Isparta region of Turkey. One hundred sixty nine samples were taken from the leaves showing virus symptoms in the vegetation period and 109 samples were taken from the tubers of suspicious plants in the harvest period. Total of 278 samples were tested by using double antibody sandwich- enzyme linked immunosorbent assay (DAS-ELISA) method. The DAS-ELISA analysis revealed that both tubers and leaves were infected with PVY, PVX, PVS, PVA and PLRV. It was determined that 87.45% (244 samples) of the tested samples were infected with one or more viruses and 12.54% (34 samples) of them gave negative reaction with DAS-ELISA. Regarding the prevalence of viruses among the potato varieties in this study, it was found that all samples belonging to Safrane and Milva varieties were infected with one or more viruses. Besides, other potato varieties showed different rates of virus infection. In the mechanical inoculation tests, serious stunting, systemic chlorosis and leaf deformation symptoms were observed on N. glutinosa, while symptoms such as mottling, leaf distortion, chlorotic and necrotic local lesions were observed on the leaves of other test plants.

Keywords: Detection, Solanum tuberosum, Virus diseases.

INTRODUCTION

Potato (Solanum tuberosum L.) is one of the most important industrial crops and human food staples in the world. Potato cultivation has been officially encouraged in Turkey since 1872, and today the country is the Middle East's biggest producer after Iran, with an output of almost 4.8 million tons in 2012 (Anonymous, 2014). The Anatolian central plateau -with its hot, dry summers and cold winters- is the most important cultivation area, accounting for nearly half of the national potato area. Turkey produces 1.3% of the total potato production of the world. Potato can be grown in almost all parts of the country. Turkey is one of the largest potato producing countries in the Mediterranean region and has a demand for 450,000 t of seed per year. Only around 50,000 t of classified seed is produced in Turkey itself, and more than 80% of Turkish potato crop is grown from farm-saved seed (Bostan and Haliloğlu, 2004).

As for all agricultural crops, plant protection problems such as diseases are the major factors decreasing potato production. The numbers of viral infections increases and they cause important production and quality losses in potato production around
the world. Over 40 viruses have been recorded as naturally infecting potato, some of which are restricted to a certain geographical region, while others occur worldwide (Jeffries, 1998). PLRV, PVY, PVA, PVX, PVM and PVS are the most common and important viruses in terms of distribution and effect on yield.

Most of the potato viruses are transmitted by aphids (Brunt, 2001). In addition, they can be transmitted via infected seed tubers. Potato viruses can cause heavy yield losses and are a serious threat to potato production (Valkonen, 2007). 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 to region by means of infested tubers (Hooker, 1986).

PLRV, PVY, PVA, PVX, PVM, and PVS are affecting potato crops singly or in combination. Yield reduction by these important potato viruses is usually higher than 50% in most susceptible cultivars (Salazar, 2003). Furthermore, potato viruses may directly affect potato quality since infected plants usually produce smaller tubers, and decrease in starch content has a negative impact on the crop nutritional value. Some viruses cause external and internal tuber necrosis, making them unsuitable for marketing (Beczner et al., 1984). Such potato viruses cause degeneration, which requires the regular replacement of the seed to maintain quality and productivity (Ali et al., 2008). It is quite obvious that detection and identification of potato viruses is a critical part of the management of seed potato production and the development of modern virologic control techniques is a most urgent practical need of the original, elite, and reproductive potato seed production (Ryazantsev et al., 2008).

Virus diseases can often be diagnosed by mosaic patterns on leaves, stunting of the plant, and leaf and tuber malformations. Symptoms are not always expressed due to interactions between the viruses and growing conditions such as fertility and the weather or the age of the plant when it is infected. Serology and nucleic acid detection techniques are often used to diagnose and characterize suspected virus diseases.

PLRV, the type member of the genus Polerovirus, and the potyviruses, PVY and PVA, belonging to family Potyviridae, are undoubtedly the most important viruses of the crop (Salazar, 2003). One factor that contributes to their importance is that they are readily transmitted by several species of aphids, common pests of potatoes. PLRV is transmitted in nature in a persistent manner by several aphid species, in particular, Myzus persicae Sulz, the most important vector. The virus survives mainly in infected volunteer potatoes and in wild hosts, although it appears that the importance of wild hosts for survival and spread is higher in tropical countries. PVY and PVA are 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). However, several other strains or isolates with particular characteristics have caused outbreaks in potato in the last 10 years. The most damaging at present is PVYN that causes ringspots on the tubers (Beczner et al., 1984).

PVX is the type member of the genus Potexvirus (Salazar, 2003). Plants often do not exhibit symptoms, but the virus can cause symptoms of chlorosis, mosaic, decreased leaf size, and necrotic lesions in tubers. PVX can interact with PVY and PVA to cause more severe symptoms and yield losses than either virus alone. The source of this virus is infected tuber material. It is transmitted mechanically, not by an insect vector. Tobacco, pepper, and tomato can also serve as hosts of PVX (Partridge, 2008).

PVS is an important problem in potato. It remained unknown until the 1950's because
its symptoms are inconspicuous. PVS can cause yield loss up to 20%. Seed potatoes are not yet certified for PVS, which contributes to its widespread distribution. Most potato cultivars are symptomless. On some cultivars, if infected early in the season, slight deepening of the veins, rough leaves, more open growth, mild mottling, bronzing, or tiny necrotic spots on the leaves can be seen. PVS is a member of the genus *Carlavirus* and is nonpersistently transmitted by aphids, including *M. persicae*. It is also mechanically transmissible and transmissible through tubers (Burrows and Zitter, 2005).

Some potato viruses have been reported from different potato growing areas in Turkey (Arlı-Sökmen et al., 2005; Bostan and Açıkgöz, 2000; Bostan and Hallıoğlu, 2004; Çtır, 1982; Güner and Yorgancı, 2006; Yılmaz et al., 1990). Despite many studies on potato viruses, data on the incidence and presence of these viruses on different potato varieties in Turkey is insufficient. Therefore, this study was conducted to detect and identify PLRV, PVY, PVA, PVX and PVS on different varieties via biological and serological methods and to determine the incidence and presence of virus diseases in the collected samples.

### MATERIALS AND METHODS

Potato seed tubers of different varieties were obtained from producers during 2009-2010 in Afyon region and were planted in the trial plots of the research center of the Faculty of Agriculture, Suleyman Demirel University, Isparta, Turkey. Leaf samples (169) showing virus symptoms were collected in the vegetation period before harvest, and tuber samples (109) at harvest time. During surveys, virus suspected plants were photographed in the trial parcels. Symptoms of plants were recorded before putting leaf samples into plastic bags and storing in a freezer at -20°C until DAS-ELISA tests were done. All samples collected from nine potato varieties were tested for the presence of viruses by using specific DAS-ELISA detection kits (Agdia, positive and negative controls, USA) PLRV, PVY, PVA, PVX and PVS. DAS-ELISA method was performed according to Clark and Adams (1977). Absorbance values were measured at 405 nm with a microplate reader (ELx800 Universal Microplate Reader, Bio-Tek Instruments Belgium). Samples with DAS-ELISA values at least twice those of the healthy control were considered as positive (Clark and Adams, 1977).

DAS-ELISA positive PVY samples were inoculated to *Chenopodium amaranticolor*, *C. quinoa*, *Vigna sinensis*, *Nicotiana tabacum* cv. Maden, *N. tabacum* cv. White Burley and *N. tabacum* cv. Xanthii and *N. glutinosa* test plants. As a result of mechanical inoculation studies conducted for other viruses are highly specific symptoms could not be obtained.

### RESULTS AND DISCUSSION

A total of 278 plant samples belonging to nine potato varieties were collected from the trial parcels in Isparta, Turkey, during the surveys. Potato varieties and number of samples used in the study are shown in Table 1. In this study, the following symptoms were observed: mosaic patterns on leaves, malformations, veinal necrosis,
chlorosis and stunting (Figures 1, 2, and 3). These symptoms on potato plants were similar to previous reports for potato viruses (Brunt, 2001; Stevenson et al., 2001). All leaves and tubers were tested by DAS-ELISA for PLRV, PVY, PVA, PVX and PVS. The result of serological tests showed that potato plants were infected with these viruses. The tested samples (244 samples) were infected with one or more viruses (87.45%) and 12.54% (34 samples) of them gave negative reaction to DAS-ELISA. Individual detection rates of the tested viruses, namely, PVY, PVS, PVX, and PVA were 13.30, 12.94, 1.43, and 0.35%, respectively. PLRV was only found as mixed infections with other viruses. Various combinations of mixed infections were determined in the tested samples. The most common mixed infection was PVY+PVS (32.73%). Other common combinations were: PVY+PVA+PVS (5.39%), PVX+PVS+PVY (5.03%), PVX+PVS (5.39%), PVA+PVY (2.51%), PVX+PVY (1.43%) and there were also other rare combinations (Table 2). Occurrence and distribution rates of PLRV, PVY, PVA, PVX and PVS in potato samples collected during surveys from trial parcels in Isparta are shown in Table 2. As a result of ELISA tests, PVY, PVS, PVX and PVA were detected in 13.30, 12.94, 1.43 and 0.35%, respectively. PLRV was not detected in the samples as a single infection. Out of the 278 samples, 127 had double virus infections and the most common double infection was PVY+PVS (32.73%). In addition, 36 samples had triple virus infections. The results of ELISA showed that three samples were infected with four viruses.

As a result of the ELISA tests, PVY and PVS were detected in 17.75 and 2.95%, respectively, in potato leaf samples. PLRV, PVX and PVA were not detected as single infection. The most common double infection was PVY+PVS (47.93%) (Table 3).

According to the results of ELISA tests, PVS, PVY, PVX and PVA were detected in 28.44, 6.42, 3.66, and 0.91% in potato tuber samples, respectively. PLRV was not detected as a single infection. The most common double infections in tubers were PVX+PVS (11.00%) and PVY+PVS (9.17%) (Table 4).

Regarding the prevalence of viruses in different varieties, all samples belonging to Safrane and Milva varieties were found to be infested with one or more viruses. Besides, infestation rates of the other varieties with
Table 2. Occurrence of viruses in potato samples collected during surveys.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Number of Samples</th>
<th>PXX</th>
<th>PYY</th>
<th>PVS</th>
<th>PVA</th>
<th>PLRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floris</td>
<td>32</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Safran</td>
<td>33</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Solea</td>
<td>33</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Úniversa</td>
<td>29</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Milva</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proventa</td>
<td>30</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Marabel</td>
<td>25</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lady</td>
<td>29</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vangogh</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>278</td>
<td>4</td>
<td>37</td>
<td>36</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Infection Rate (%)  
|                  | 1.43 | 13.30 | 12.94 | 0.35 | 0.00 | 1.43 | 32.73 | 2.51 | 5.39 | 1.07 | 0.35 | 0.35 | 1.43 | 5.03 | 5.39 | 0.35 | 0.35 | 1.79 | 0.71 | 0.35 |


Table 3. Occurrence of viruses in potato leaves collected from potato varieties during surveys.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Number of Samples</th>
<th>PXX</th>
<th>PYY</th>
<th>PVS</th>
<th>PVA</th>
<th>PLRV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floris</td>
<td>20</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Safran</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Solea</td>
<td>20</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Úniversa</td>
<td>19</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Milva</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proventa</td>
<td>19</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Marabel</td>
<td>13</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lady</td>
<td>18</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vangogh</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
<td>0</td>
<td>30</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Infection Rate (%)  
|                  | 0.00 | 17.75 | 2.96 | 0.00 | 0.00 | 1.18 | 47.93 | 3.55 | 1.78 | 1.78 | 0.59 | 2.37 | 3.55 | 8.28 | 0.59 | 0.59 | 2.96 | 0.59 | 0.59 |

viruses were found in Floris, 94.28% for Vangogh, 86.70% for Universa, 84.84% for Solea, 83.33% for Proventa, 72.00% for Marabel, and 65.51% for Lady. This is the first study dealing with the infestation rates of different potato varieties with potato viruses in this region.

When PVY was inoculated to C. amaranticolor, C. quinoa and Vigna sinensis test plants, some symptoms such as mottling, leaf distortion, chlorotic and necrotic local lesion were observed on leaves. Severe stunting, systemic chlorosis, and leaf deformation were observed on N. glutinosa (Figure 4), while N. tabacum cv. Maden, N. tabacum cv. White Burley and N. tabacum cv. Xanthi did not show any symptoms. These symptoms were similar to those that were described previously for PVY (Hooker, 1986; Güner and Yorgancı, 2006).

These viruses have previously been detected in other regions of the country; however, this is the first time that they were determined in this region of Turkey. Çıtır et al. (1999) found that PVX was prevalent in Tokat province. In the survey conducted in Van province for potato viruses, PVX and PVY were found as single infections, whereas PLRV and PVA were detected as mixed infections in potato growing areas of Nigde and Nevşehir provinces. By using serological and biological methods, they found PVY, PVS, PVA, PVA and PLRV as single or mixed infections in potato leaves and tubers by using DAS-ELISA method in Nigde and Nevşehir provinces. Büşkan and Haliloglu (2004) found PLRV when PVY was inoculated to C. amaranticolor, C. quinoa and Vigna sinensis test plants in Van province. During 2001-2004, Güler and Yorgancı (2006) performed research to detect viral pathogens in potato growing areas of Nigde and Nevşehir provinces by using serological and biological methods. They found PVY, PVS, PVA, PVA and PLRV as single or mixed infections in potato leaves and tubers by using DAS-ELISA method in Nigde and Nevşehir provinces. When PVY was inoculated to C. amaranticolor, C. quinoa and Vigna sinensis test plants, some symptoms such as mottling, leaf distortion, chlorotic, and necrotic local lesion were observed on leaves. Severe stunting, systemic chlorosis, and leaf deformation were observed on N. glutinosa (Figure 4), while N. tabacum cv. Maden, N. tabacum cv. Xanthi did not show any symptoms. These symptoms were similar to those that were described previously for PVY (Hooker, 1986; Güner and Yorgancı, 2006).
(13.28%), PVS (6.4%), PVX (6.9%) and PVY (16.8%) in seed tubers in important potato growing provinces.

In this study, all the potato varieties were found to be infected with one or more viruses. The result of ELISA showed that the most common viruses in the collected samples were PVS and PVY. These viruses are known to be transmitted mechanically, by contact with diseased plants in nature, through tubers, and by aphids (Hooker, 1986; Burrows and Zitter, 2005). The occurrence and wide distribution of PVY and PVS 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 the management of 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 is necessary for virus-free potato production.

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