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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*, Potyvirus (PVY), *Potato virus X*, Potexvirus (PVX), *Potato virus S*, Carlavirus (PVS), *Potato virus A*, Potyvirus (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 Mediterrean 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

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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 (PVY^O, PVY^N and PVY^C). 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 PVY^N 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 Haliloğlu, 2004; Çıtır, 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 spesific 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,

Table 1. Potato varieties and number of samples used in the study.

Potato Varieties	No of leaf samples	No of tuber samples
Solea	20	13
Safran	20	13
Floris	20	12
Proventa	19	11
Milva	20	12
Üniversa	19	10
Lady olympia	18	11
Vangogh	20	15
Marabel	13	12
Total	169	109

Figure 1. Mosaic, chlorosis, decreased leaf size and leaf malformations on a potato plant.

Figure 2. Necroses on a leaf of a potato plant.

Figure 3. Stunting of potato plants.

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.

Varieties	Number of Samples	PVX[a]	PVY[b]	PVS[c]	PVA[d]	PLRV[e]	PVX PVY	PVY PVS	PVY PVA	PVX PVS	PLRV PVY	PLRV PVX	PLRV PVA	PLRV PVS	PVS PVA	PVX PVY PVS	PVY PVA PVS	PLRV PVS PVA	PVX PVY PVA	PLRV PVY PVS	PVX PVY PVA PVS	PLRV PVS PVY PVX
Floris	32	1	5	1	0	0	0	6	1	4	1	1	0	0	0	5	4	1	1	0	0	0
Safran	33	1	2	6	0	0	0	10	1	4	0	0	0	0	0	3	3	0	0	2	1	0
Solea	33	1	6	4	0	0	0	11	0	2	1	0	0	0	0	2	0	0	0	1	0	0
Üniversa	29	0	1	6	0	0	0	12	1	0	0	0	0	0	0	3	2	0	0	0	0	0
Milva	32	0	0	7	0	0	0	22	0	0	0	0	0	0	1	0	1	0	0	0	1	0
Proventa	30	0	13	1	1	0	2	5	1	0	0	0	1	0	0	0	1	0	0	0	0	0
Marabel	25	0	5	2	0	0	1	4	1	2	1	0	0	0	0	0	0	0	0	1	0	1
Lady	29	1	5	1	0	0	1	8	2	0	0	0	0	0	1	0	0	0	0	0	0	0
Vangogh	35	0	0	8	0	0	0	13	0	3	0	0	0	1	2	1	4	0	0	1	0	0
Total	278	4	37	36	1	0	4	91	7	15	3	1	1	1	4	14	15	1	1	5	2	1
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	0,35	1,43	5,03	5,39	0,35	0,35	1,79	0,71	0,35

[a] *Potato virus,* Potyvirus , [b] *Potato virus Y,* Potyvirus, [c] *Potato virus S*, Carlavirus, [d] *Potato virus A*, Potyvirus, [e] *Potato leafroll Luteovirus.*

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

Varieties	Number of Samples	PVX[a]	PVY[b]	PVS[c]	PVA[d]	PLRV[e]	PVX PVY	PVY PVS	PVY PVA	PVX PVS	PLRV PVY	PLRV PVX	PVS PVA	PVX PVY PVS	PVY PVA PVS	PLRV PVS PVA	PVX PVY PVA	PLRV PVY PVS	PVX PVY PVA PVS	PLRV PVS PVY PVX
Floris	20	0	3	0	0	0	0	6	1	0	1	1	0	2	4	1	1	0	0	0
Safran	20	0	1	2	0	0	0	9	1	1	0	0	0	0	3	0	0	2	1	0
Solea	20	0	5	1	0	0	0	10	0	1	1	0	0	1	0	0	0	1	0	0
Üniversa	19	0	1	1	0	0	0	12	1	0	0	0	0	2	2	0	0	0	0	0
Milva	20	0	0	1	0	0	0	17	0	0	0	0	1	0	1	0	0	0	0	0
Proventa	19	0	11	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0
Marabel	13	0	4	0	0	0	0	4	1	1	1	0	0	0	0	0	0	1	0	1
Lady	18	0	5	0	0	0	1	7	2	0	0	0	1	0	0	0	0	0	0	0
Vangogh	20	0	0	0	0	0	0	12	0	0	0	0	2	1	4	0	0	1	0	0
Total	169	0	30	5	0	0	2	81	6	3	3	1	4	6	14	1	1	5	1	1
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

[a] *Potato virus,* Potyvirus , [b] *Potato virus Y,* Potyvirus, [c] *Potato virus S*, Carlavirus, [d] *Potato virus A*, Potyvirus, [e] *Potato leafroll Luteovirus.*

Table 4. Occurrence of viruses in potato tubers collected during surveys.

Varieties	Number of Samples	PVX[a]	PVY[b]	PVS[c]	PVA[d]	PLRV[e]	PVX PVY	PVX PVS	PVY PVS	PVY PVA	PLRV PVA	PLRV PVS	PVX PVY PVS	PVY PVA PVS	PVX PVY PVA PVS
Floris	12	1	2	1	0	0	0	4	0	0	0	0	3	0	0
Safran	13	1	1	4	0	0	0	3	1	0	0	0	3	0	0
Solea	13	1	1	3	0	0	0	1	1	0	0	0	1	0	0
Üniversa	10	0	0	5	0	0	0	0	0	0	0	0	1	0	0
Milva	12	0	0	6	0	0	0	0	5	0	0	0	0	0	1
Proventa	11	0	2	1	1	0	1	0	1	1	1	0	0	1	0
Marabel	12	0	1	2	0	0	1	1	0	0	0	0	0	0	0
Lady	11	1	0	1	0	0	0	0	1	0	0	0	0	0	0
Vangogh	15	0	0	8	0	0	0	3	1	0	0	1	0	0	0
Total	109	4	7	31	1	0	2	12	10	1	1	1	8	1	1
Infection Rate (%)		3,67	6,42	28,44	0,92	0,00	1,83	11,01	9,17	0,92	0,92	0,92	7,34	0,92	0,92

[a] *Potato virus*, Potyvirus , [b] *Potato virus Y*, Potyvirus, [c] *Potato virus S*, Carlavirus, [d] *Potato virus A*, Potyvirus, [e] *Potato leafroll Luteovirus*.

viruses were found as 96.00% for 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. Xanthii 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 with PVX+PVY (Demir *et al*., 2001). During 2003-2004, Güner and Yorgancı (2006) performed research to detect viral pathogens in potato growing areas of Niğde and Nevşehir provinces, by using serological and biological methods. They found PVY, PVS, PVX, PVA and PLRV viruses as single or mixed infections in both potato leaves and tubers by using DAS-ELISA method in Niğde and Nevşehir provinces. Bostan and Haliloğlu (2004) found PLRV

Figure 4. Stunting and leaf deformation in *N. glutinosa* (Left: Inoculated with PVY, Right: Healthy plant).

(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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شناسایی ویروس های PVY، PVX، PVS، PVA، و PLRV روی رقم های مختلف سیب زمینی در ترکیه با استفاده از DAS-ELISA

ن. یاردیمسی، ه. شلال کلیش، و ی. دمیر

چکیده

هدف این پژوهش مطالعه و تشخیص ویروس وای سیب زمینی (PVY)، ویروس ایکس سیب زمینی، پوتکس ویروس (PVX)، ویروس اس سیب زمینی، کارلاویروس(PVS)، ویروس ای سیب زمینی ، پوتی ویروس(PVA)، و ویروس برگ قاشقی سیب زمینی (PLRV) روی غده و برگ رقم های مختلف سیب زمینی بود. این رقم ها شامل Solea، Safran، Floris، Proventa، Milva، Universa، Lady olympia، Vangogh، و Marabel بود که در منطقه افیون در ترکیه کشت می شدند. به این منظور، در طی ۲۰۱۰-۲۰۰۹ ، غده های سیب زمینی از تولیدکنندگان منطقه افیون تامین شد و در کرت های آزمایشی در منطقه ایسپارتا در ترکیه کشت شد. در اجرای آزمایش، ۱۶۰ نمونه برگ که در دوره رشد سبزینه ای علایم آلودگی ویروسی را نشان میداد و نیز ۱۹۴ نمونه غده های بوته های مشکوک در هنگام برداشت تهیه شد. در مجموع، ۲۷۸ نمونه با روش داس الیزا (DAS-ELISA)ارزیابی شد. این روش تجزیه آشکار ساخت که برگ ها و غده ها هر دو به ویروس های PVY، PVX، PVS، PVA، و PLRV آلوده بودند. نیز معلوم شد که ۸۷.۴۵٪ (۲۴۴ نمونه) از نمونه های آزمون شده به یک یا چند ویروس آلوده بودند و ۱۲.۵۴٪ از نمونه ها در آزمون داس الیزا نتیجه منفی داشتند. در ارتباط با فراوانی و آلودگی ویروسی در رقم های سیب زمینی در این پژوهش، چنین دریافت شد که همه نمونه های رقم های Safrane و Milva به یک یا چند ویروس آلوده بودند. همچنین، دیگر رقم ها درجات آلودگی مختلفی نشان دادند. در آزمون های تلقیح مکانیکی ، کاهش شدید رشد وکوتولدگی بوته، کلروز سیستمیک و تغیر شکل برگ توتون *N. glutinosa* مشاهده شد در حالیکه روی برگ دیگر گیاهان آزمون شده، علایمی مانند پیسک، خمیدگی یا پیچیدگی برگ و لکه های موضعی کلروز یا مرده به چشم می خورد.