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

N. Yardımci, H. Çulal Kılıç and T. Özdemir

Süleyman Demirel University Faculty of Agriculture Department of Plant Protection, Isparta 32260
Corresponding author, Email address: handankilic@sdu.edu.tr

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 PYV. As a result of RT-PCR, 23 potato leaf samples (44.23 %) 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 annuum* 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 (Kerlan 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 (PVY^O, PVY^N and PVY^C). The most damaging one at present is PVY^N that causes ringspots on the tubers. In addition to transmitted by aphids, it can be transmitted via infected seed 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 to region by means of infested tubers (Burrows and Zitter, 2005).

PVY is a member of the family *Potyviridae* with non-enveloped filamentous, flexuous rods 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 (Kerlan, 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,

Bostan and Haliloğlu, 2004, Güner and Yorgancı, 2006), but this is the first study which detected PVY using biological and RT-PCR method, from the Southwest region in Turkey. We have initiated virus screening program to determine the common viral disease in potato fields in the region.

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 -20^0 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. H_2O was used as a negative control. In RT-PCR study, a sample yielding positive results in previous ELISA test was used as positive control (Yardımcı *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 µl reaction mixture containing, 21µl H_2O, 25 µl 2x1 PrimeScript One Step RT-PCR buffer (containing dNTP mixture, One step Enhancer solution), 2 µl Prime Script 1 step enzyme mix, 1 µl 20 Mm primers. Oligonucleotide primer sequences reported by Shalaby *et al*., (2002) were used to detect PVY.

Primer I: 5'
TCAAGGATCCGCAAATGACACAATTGATGCAGG 3'
Primer II: 5'
AGAGAGAATTCATCACATGTTCTTGACTCC 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: 50ºC for 30 min., 94ºC for 2 min., then 30 cycles of 94ºC for 30 second, 55ºC for 30 second and 72ºC for 1 min., followed by 72 ºC for 3 min. PCR products were separated in 1% agarose gel by electrophoresis, stained with 0.5 µg/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.

Mechanical inoculation studies: RT-PCR-positive leaf samples were used as inoculum. PVY-infected potato leaves were prepared in a phosphate buffer (0.01 M, pH 7.2, 1 ml per 1g of leaf material) and were applied to the indicator plants. *Chenopodium amaranticolor, C. quinoa, Nicotiana glutinosa*, *N. tabacum* White Burley and *N.tabacum* Xanthii were used as the test plants. The inoculated plants were grown in a greenhouse at 22ºC-28ºC. The occurrence and type of symptoms were observed on inoculated leaves after inoculation. In addition showing systemic infections *Nicotiana glutinosa* plants were tested by RT-PCR.

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 surveys 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 amaranticolor, 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.

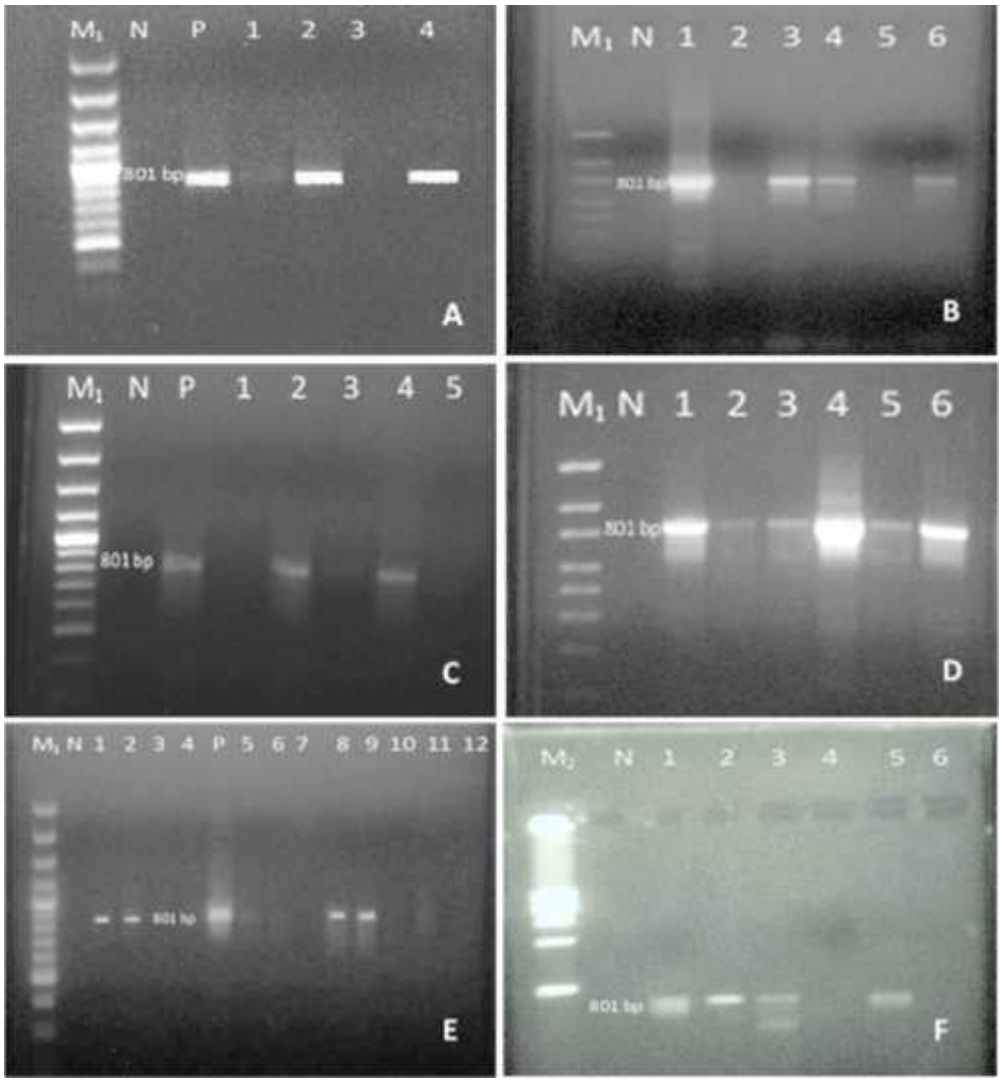

Figure 2. A.B.C.D.E.F. Agarose gel electrophoretic stained by ethidium bromide analysis of RT-PCR amplification of PVY cp gene from infected field samples of potato. M_1: Markır 100 bp DNA ladder, M_2: Markır 1 kb DNA ladder, P: Positive control, N: Negative control, Lanes: infected field samples.

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.

Name of variety	Collected samples	Infected samples	Disease ratio (%)
Universa	13	7	53.84
Marabel	6	4	66.66
Milva	5	2	40.00
Solea	10	5	50.00
Vangogh	3	1	33.33
Floris	5	2	40.00
Safran	5	1	20.00
Proventa	5	1	20.00
Total	52	23	44.23

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