

Field Survey and Molecular Detection of Potato Viruses in Northern Fields of Palestine

مسح ميداني وكشف جزيئي لفيروسات البطاطا في حقول الشمال من فلسطين

Raed Alkowni* & Hassan Abu-Qaoud**

راند الكوني، وحسان أبو قاعود

*Biology & Biotechnology Department, Faculty of Science,

**Plant Production & Protection Department, Faculty of Agriculture,

An-Najah National University, Nablus, Palestine.

*Corresponding author, Email: ralkowni@najah.edu

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Abstract

Sanitary status of potatoes grown in Northern fields of Palestine; based on their viral infections, was assessed biologically and molecularly in this study. Visual inspections for symptomatic disorders were carried during the growing seasons of 2013-2014, reporting a leaf curling, yellowing and stunting plant syndromes. Molecularly, two of most economically damaging viruses (Potato virus Y and Potato virus X) were detected by reverse transcriptase polymerase chain reaction (RT-PCR), with total infection (9.5%). PVY but not PVX, was found in several locations and cultivars. Beside sanitary assessment of potato, this is the first to report the detection of both potato viruses (PVX and PVY) molecularly in Palestinian fields.

Key words: Potato virus; Palestine; RT-PCR; PVY; PVX.

ملخص

في هذه الدراسة واستنادا إلى قياس نسب الإصابة بالفيروسات، جرى أول تقييم بيولوجي وجزيئي للوضع الصحي لمحصول البطاطا المزروعة في حقول الشمال من فلسطين، حيث

اجريت عدة زيارات حقلية للكشف عن اية اعراض مرضية على المحصول خلال مواسم النمو من 2013-2014، وقد لوحظ وجود اعراض امراض فيروسية مثل التقاف الورق، والاصفرار، واعراض تقزم النبات. جزيئيا، تم الكشف عن اثنين من أكثر الفيروسات الضارة اقتصاديا (فيروس البطاطس Y وفيروس البطاطا X) من خلال فحص مبلمرة سلسلة الحمض النووي باستخدام تقنية (RT-PCR)، لتظهر نسبة الإصابة بالفيروسات (9.5%). وقد تم العثور على فيروس البطاطس Y في عدة مواقع وأصناف بعكس فيروس البطاطا X الذي انحصر في صنف واحد هو (الاسكا). تعتبر هذه الدراسة الاولى في فلسطين للإبلاغ عن الكشف عن تلك الفيروسات من خلال الفحص الجزيئي، بجانب كونها اول عملية تقييم لصحية نبات البطاطا، المزروع في حقول شمال فلسطين.

الكلمات المفتاحية: فيروسات البطاطا، فلسطين، مبلمرة سلسلة الحمض النووي، (فيروس البطاطس Y وفيروس البطاطا X).

Introduction

Potato (*Solanum tuberosum* L.); one of the important cash crops worldwide, is affected by several pests and pathogens that causing significant yields losses (Oerke, 2006). More than 35 viruses and virus like pathogens were isolated from infected potatoes (Salazar, 1996; Wang, & *et al.*, 2006; Loebenstein & Gaba, 2012) Potyvirus: *Potato virus Y* (PVY); Polerovirus: *Potato leafroll virus* (PLRV), Carlavirus: *Potato virus S* (PVS), and Potexvirus: *Potato virus X* (PVX) were the most known devastating viruses.

The *Potyviridae* comprises almost 20% of classified plant viruses. PVY; a family member, was reported to cause up to 90% losses in crops yields (Beczner, & *et al.*, 1984; Oerke, & *et al.*, 1994; Salazar, 1996). Their genomes consist of positive single-stranded RNA (10 kb); with a small protein at 5' end and poly(A)_n sequence at 3' end (Shukla, & *et al.* 1994). Members of this genus sharing some highly conserved regions of genomes where made it possible to develop universal primers for their detection from plants (Gibbs & Mackenzie, 1997; Langeveldt, & *et al.* 1991; Pappu, & *et al.* 1993; Chen, & *et al.* 2001). In fact, PVY was one of top ten studied viruses' worldwide (Scholthof, & *et al.* 2011). PVY was found extremely variable in nature (El-Absawy, & *et al.* 2012), and strains (PVY^O, PVY^N and PVY^C) were recognized (de Bokx & Huttinga, 1981). The most worldwide occurred strain is PVY^O.

Potato virus X (PVX), a type member of the genus *Potexvirus* within the family *Flexiviridae* (Adams, & *et al.* 2004; Smith, 1931), is not transmitted by any insect vector (Salazar, 1996). PVX can cause more severe symptoms and yield loss in mixed infections with PVY and PVA (Jayasinghe, & *et al.* 1989).

Serological assay (e.g. ELISA) is most common method of virus detection (Shojaei, & *et al.* 2009); however, the molecular tool (e.g. RT-PCR), is sensitive and could be used for measuring the variations in RNA viruses (Klerks, & *et al.* 2001).

In Palestine, potatoes are one of strategic and commercial crop. With tomatoes and squash, it was occupying the majority of vegetables planted areas (10 – 15%). Potatoes mostly found where water for irrigation is abundant (FAO, 2001).

This study aimed to investigate and assess the occurrences of potato infecting virus diseases biologically and molecularly, in order to state the sanitary status of planted potatoes in Palestinian fields, from viral disease point of view, for the first time in the State of Palestine.

Materials and methods

Field surveys and samples collection. Field surveys were carried in Northern areas of Palestine (Fig. 1) where potato was extensively cultivated in Tubas, Nablus and Jenin districts. Several visits to these fields were achieved in the period between late Autumn-2013 and early Spring-2014. Information about the cultivated potatoes and viral symptoms were firstly reported from growers. Following transect line random sampling method (Krebs, 1999), plants out randomly selected furrow lines of each field were inspected and potato plants on each line were inspected. About 16 fields distributed over these areas were subjected to investigations and 63 samples were then collected randomly from symptomatic and asymptomatic potatoes plants leaves. Collected potato samples were kept at 4°C for molecular tests.

RNA extraction and purification. Total nucleic acids and RNA purification of potato samples were done using the extraction methods of

RNA PureLinke RNA Mini Kit (Life Technologies Corporation, Carlsbad, CA, USA) and following the manufacturer protocols (Rump, & *et al.* 2010). Briefly; 0.5 g of (fresh) potato tissues selected from different parts of plant, was grinded in the extraction buffer (lysis buffer with 1% mercaptoethanol), and following PureLinke RNA Mini Kit recommended protocol. The extracts were purified by maximum speed centrifugation for 10 min. After that, one volume of 70% ethanol was added to collected 500µl of the supernatant before the mix was transferred to a spin cartridge column. Several washes by 700µl of the washing buffer-I, the spin cartridge was placed into a new collection tube. Two washings with the buffer-II were carried by 12000 rpm centrifugation for 15 sec at room temperature. The fluids in the collection tube were then discarded. The spin cartridge was centrifuged at 12000 rpm for 2 min to dry the membrane with bound RNA. To elute the RNA from the membrane, 60µl of RNase-free water was added to the centre of the spin cartridge and incubated at room temperature for 1 min before 2 min centrifugations. Purified RNAs were viewed in gel electrophoresis which was run in 1xTBE for quality control assurance. The purified RNAs were stored at -80°C for later use in RT-PCR tests.

Reverse Transcription (First Strand cDNA synthesis). cDNAs were synthesized by using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT; Promega Corporation, USA). Briefly, 3µl of previously purified RNAs were mixed with 0.3µl of Random Hexamer Primers [d(N)₆] in 2µl of 10X buffer. Then the mix was put in a hot bath (70°C) for 10 min and then left at room temperature for 15 min. Then the following mixture was added: [1µl dNTPs (10mM); 2µl DTT (0.1 M) 0.5µl RNase inhibitor; 1µl M-MLV]. Then the final volume was adjusted to 20µl with sterile distilled water. Then the mixture was kept at 37°C for 1 hr, before it was incubated on ice for immediate use or at -20°C for conservation.

Polymerase chain reaction (PCR) amplifications and analysis. Specific primers able to amplify about 801bp from the PVY coat protein were selected as recommended (Al-Taleb, & *et al.* 2011). These primers were named as PVY-Forward: 5'-GCAAATGACACAATTGATGCAG-3'

(40% GC content) and PVY-Reverse: 5'-CATCACATGTTCTTGACTCC-3' (45% GC content). Other set of primers was selected, able to amplify specifically 562 bp from the PVX coat protein as recommended (Nie, and Singh, 2000; Bostan and Peker 2009). The primers were as follows: PVX-Forward: 5'-TAGCACAACACAGGCCACAG-3' with (55% GC content and 20 nt. length) and PVX-Reverse: 5'-GGCAGCATTCATTTTCAGCTTC-3' with (47% GC content and 21 nt. length). Primers were obtained from *Hylabs* Ltd.

A total of 2µl cDNAs were mixed with 48µl PCR mix composed of [4µl Primer1 and Primer2 (2.5µM); 5µl of 10x Thermophilic DNA buffer; 4µl MgCl₂ (25mM); 1µl dNTPs (10mM); 2.5µl Taq DNA polymerase (2u\µl); and total volume (50µl) was adjusted using sterile distilled water]. Then the mixture was put in PCR machine (Biometra GmbH, Germany) at (94°C Denaturation / 30 sec; 55°C Annealing / 30 sec; 72°C Extension / 1 min) for 35 cycles. The final extension was at 72°C / 10 min.

PCR products were analyzed in 1.2% agarose gel electrophoresis (Labnet International Inc., USA) running in 1x TAE buffer and for 40 min at 100 volt. The gels were observed on UV-documentary system (MultiDoc-It™ 120 Digital Imaging System, UVP, USA) after Ethidium bromide staining (1mg/ml) for 5 min at room temperature.

Results and Discussion

Biological assays: Palestinian fields are usually planted with potatoes in two growing seasons: Autumn/Winter and Winter/Spring, in areas where irrigation waters are available and abundant. It was hardly to notice any growth abnormalities in the randomly surveyed furrow lines of many fields, indicating the resistance quality of potato cultivars which used by farmers. In fact, the planted potato cultivars in north Palestine were (*Solanum tuberosum* cv. Mondial) and (*Solanum tuberosum* cv. Spunta) and were mainly imported from international seed companies (mainly from Netherlands and throughout Israeli importing companies). Some potato plants exhibited growth abnormalities, leaf curling's, yellow leaves, or stunted plants, putatively virus disease symptoms (Fig. 2).

Collectively, plants stunting and leaves curling were observed in less than 1% of the surveyed plants, meanwhile leaves yellowing were on about 4.5% of inspected potato plants and always following transect line random sampling method (Krebs, 1999) on each inspected lines. These observations were noticed on both growing seasons.

Since potyviruses (including PVY) were known to be transmitted by several aphid vectors (Fereset, *et al.* 1993); none of aphid colonies were noticed on planted potatoes during the achieved surveys. This might be referred to unfavorable weather conditions at that period of the year and intensively use of pesticides.

The field inspection exhibited cultivars resistance to viral infections. This was in agreements with cultivars reported properties (NIVAP, 2015), that mentioned potato cultivar Mondial had resistance to *Potato virus X* (High - Very high) and resistance to *Potato virus Y* (Medium - High), meanwhile the potato cultivar Spunta possessed (Medium – High) resistance to PVX and (High) resistance to PVY. Knowing their high rate of genetic variations (Malpica, 2002; Alkowni, & *et al.* 2011), RNA viruses could emerge to be epidemic to the crop (i.e. *Cucumber mosaic virus* (CMV) on tomato crops in eastern Spain (Escriu, & *et al.* 2000); *Pepino mosaic virus* (PepMV) in tomatoes (Elena, & *et al.*, 2011). Regarding that, samples from symptomatic and asymptomatic potatoes at randomly basis were collected in each field, to test the presence of PVY and/or PVX, which could be in potatoes as latent infections.

Molecular Detection of PVX and PVY. Virus-specific primers in two steps RT-PCR tests were able to detect both viruses (Fig. 3). PVY was detected in 6.3% of samples (belonged to either Spunta or Mondial, but not in other cultivar such as Alaska). This relatively low incidence percentage came in agreement with the cultivar high resistance to PVY (NIVAP, 2015). Surprisingly, PVX was the only virus detected of Alaska cultivar. One fourth of Alaska’s cultivar was infected with PVX, suggesting the cultivar medium –low resistance to the virus. Due to its vulnerability to viral diseases, might be the reason for Alaska’s was less spreading potato cultivar in Palestinian fields if compared with Mondial and Spunta cultivars.

Potato virus Y (PVY) which is worldwide spreading has great economic importance, as infecting different crops such as potato, pepper, tomato, and tobacco (Fletcher, 2001; Arli-Sokmen, & *et al.* 2005). Recently it was detected in hot pepper (Sawalha, 2011) and tomatoes fields (Sawalha, 2012) in Northern Palestine, confirming the existence of the virus in this vital agricultural area. Besides, several species of weeds were considered as virus reservoirs and sources of inoculums (Latorre, 1983). These weeds-infected viruses which exhibit generally latent infections would be able to be transmitted to the potatoes mechanical contact or by vectors. PVY was reported to be transmitted through infected potato seed tubers and by at least 50 species of aphids in non-persistent manor (Ragsdale, & *et al.*, 2001). *Myzus persicae* (Sulz.) (*Hemiptera: Aphididae*) is the most efficient and common vector found in nature (de Bokx & Huttinga, 1981). In this tale, low populations of this vector in the time of planting potatoes in Northern Palestine reduce it influences on spread of the PVY, similarly founded in other cold regions (Radcliffe, 1982; Radcliffe & Ragsdale, 2002).

Potato virus X (PVX) did not exhibit symptoms of infected plants (Hameed, & *et al.* 2014), even though it may interact with other viruses such as PVY to cause more severe symptoms and yield loss rather than either virus alone. All trials to transmit the virus mechanically on 12 different herbaceous plants (Unpublished data; An-Najah Biotechnology research Lab) were failed; confirming that PVX was only transmitted mechanically. As it had no known insect vector (John, & *et al.* 2013), the PVX infected potatoes from Alaska's cultivar, suggesting the main source of this virus was the infected propagated material (potato tubers).

Conclusion

Field surveys reported few putative virus symptoms of potatoes planted in Northern Palestine. This research study was carried for the first time in the country, including molecular detection of two important potato viruses (PVX and PVY). *Potato virus Y* was detected in potatoes cultivated in Northern fields of Palestine (Spunta and Mondial), but not on Alaska. Meanwhile *Potato virus X* was only detected on Alaska cultivar. This research work recommended studying the genetic

diversities of these viruses in Palestinian fields to determine the variants. Also, assessment of the sanitary status of the potato crops in the country is inevitable demanding. Comparative study with other serological tools might be suggested. Distribution of certified-virus free healthy potato tubers was strongly advised for potato growing farmers.

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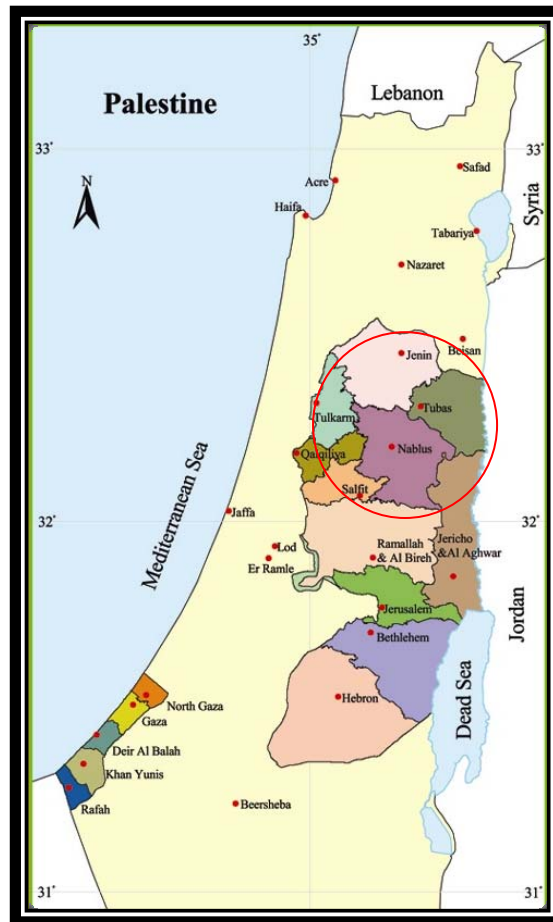


Figure (1): Map of Palestine, where the field surveys had been carried at northern part including the three main districts (Jenin, Nablus & Tubas) shown in circle. (Source: PCBS, 2014).



Figure (2): One of potato fields in Nablus district area that was surveyed in early spring 2014 to assess the sanitary status regarding to virus and virus-like diseases. Potato sample exhibiting slight leaf inter-vein yellowing (upright).

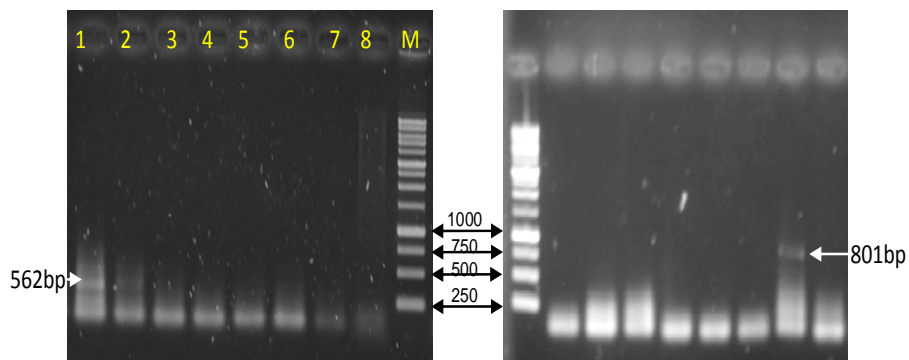


Figure (3): RT-PCR gel electrophoresis analysis for detection of *Potato virus X* (left) *Potato virus Y* (right) in samples collected from different fields in Northern Palestine using two step RT-PCR. The gel was stained by ethidium bromide and 10Kb DNA Ladder RTU was used as a DNA marker (M).