

**An-Najah National University**  
**Faculty of Graduate Studies**

**Serological and Molecular Detection of *Potato virus Y* (PVY) in West Bank**

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**2016**

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## **Dedication**

**This thesis is dedicated to:**

**The Sake of Allah, My Creator and My Master**

**My Great Parents (Yahya and Samar)**

**Who have raised me to be the strong person whom I am today and  
whose their support is always push me to the top.**

**My Sweet Sisters (Al-anood and Seham)**

**Who have provided me with a strong love shield that always surrounds  
me and never lets any sadness enter inside.**

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**Who always stay by my side and encourage me to reach my goals.**

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## الإقرار

أنا الموقع أدناه، مقدم الرسالة التي تحمل عنوان:

**Serological and Molecular Detection of *Potato virus Y* (PVY)  
in West Bank**

الكشف السيرولوجي (المصلي) والجزيئي لفيروس البطاطا واي (PVY) في الضفة الغربية

أقر بأن ما اشتملت عليه هذه الرسالة هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وإن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أية درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

### Declaration

The work provided in this thesis, unless otherwise referenced, is the research's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: Nibal Yahya Lutfi Khudiesh

اسم الطالب:

Signature:



التوقيع:

Date:

5 / 4 / 2016

التاريخ:

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## List of Abbreviations

|        |   |
|--------|---|
| Bp     | Base pair   |
| cDNA   | Complementary DNA                                 |
| CIP    | International Potato Center                       |
| CMV    | Cucumber mosaic virus                             |
| DNTPs  | Deoxyribonucleotide triphosphates                 |
| DTT    | Dithiothreitol                                    |
| EDTA   | Ethelendiaminetetraacetic acid                    |
| Mm     | Millimole   |
| M-MLV  | Moloney murine leukemia virus                     |
| μl     | Microliter  |
| No.    | Number  |
| PPV    | Plum pox virus                                    |
| PVA    | Potato virus A                                    |
| PVS    | Potato virus S                                    |
| PVV    | Potato virus V                                    |
| PVX    | Potato virus X                                    |
| RNA    | Ribonucleic acid                                  |
| ss-RNA | single-stranded ribonucleic acid                  |
| Rpm    | Round per minute                                  |
| RT-PCR | Reverse transcriptase – Polymerase Chain Reaction |
| Taq    | <i>Thermus aquaticus</i>                          |

## **Serological and Molecular Detection of *Potato virus Y* (PVY) in West Bank**

**Prepared by**  
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**Supervisor**  
**Dr. Raed Alkowni**

### **Abstract**

Potato virus Y (PVY) is widely distributed virus in potato (*Solanum tuberosum*) all over the world as ranks the fifth among the top ten of the most economically damaging plant viruses worldwide. Within the scope of collecting data of phytoviruses in Palestine, this study was conducted to reveal the incidence of PVY virus on potatoes collected from the main growing fields of potato in West Bank-Palestine, using serological methods. Besides, biological (Field inspections and mechanical inoculation) with molecular tools (RT-PCR) were used as well. A total of 255 potato leaf samples from inspected 40 fields of different regions in West Bank were collected during the 2014 / 2015 growing seasons of potato. All samples were tested for PVY by Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA), and at least 5 of these samples were further analyzed by Reverse Transcription Polymerase Chain Reaction (RT-PCR) using degenerate primers. The obtained results of the field surveys revealed a variety of symptoms such as yellowish-green mosaic, stunting, rugosity, wilting and general yellowing, which were observed in some fields; indicating the presence of putatively viral diseases. By using DAS-ELISA, the incidence of the PVY virus was detected at an average of 15.29 %; confirmed by bioassay tests and RT-

PCR analysis. Surprisingly, all infected potatoes were recorded on Spunta variety except one sample from (Mondial), meanwhile the most area where the virus had been detected in its fields found in Nablus region (48%). That presence of the virus in the fields of the main growing areas, as this study reported, should be alarming and need actions to prevent its spread. Also these results are considered important in providing a platform helpful for further studies to create proper management approach to control plant viral diseases in Palestine.

**CHAPTER ONE**  
**INTRODUCTION**

## 1.1 Introduction

"Pray for peace and grace and spiritual food for wisdom and guidance, for all these are good. But don't forget the potatoes"(9).

Potato (*Solanum tuberosum*) is a herbaceous annual plant which belongs to the *Solanaceae* or "nightshade" family of flowering plants. It was originated in the Andes Mountains of South America more than 8000 years ago. The global total crop production of potato exceeds 376 million metric tons (6). It currently ranks the world's fourth most important food crop after maize, wheat, rice, and the first among root and tuber crops (1, 2, 27). More than 6 billion people worldwide eat potato, which is produced in over 130 countries worldwide (1, 7).

Potato is well known for its high nutritional quality; it is a good source of carbohydrate, potassium, vitamins B and C. Moreover it is a source of both food and cash income. It has been known as one of the major crops to alleviate hunger in the world. The yield of potato for one hectare can return two to four times the food quantity of grain crops (13, 14, 1).

During early 1960s, the increase in potato production area has rapidly exceeded all other food crops in developing countries. It is an essential element in the food security for millions of people across South America, Africa, and Asia, including Central Asia (4, 28). From 1997 to 2007, the potato cultivation in developing countries increased by 25 percent. Nowadays, developing countries become the source of more than half of global potato production, according to International Potato Center (CIP).

## **1.2 Potato Cultivation and Production in West Bank.**

In Palestine (West Bank & Gaza Strip) cultivated potato is considered as one of the major solanaceous field crops; statistics showed that the area planted to potato utilized 24,853 dunum during 2012/2013 with an annual production of 84,175 metric tonnes (5).

In the West Bank potato is planted at two main growing seasons; the first season is in spring and the second is in fall. Farmers depend mainly on imported seed tubers particularly from Netherland. Number of farmers depends on stored parts of the spring crop to be used as seed potato for the next fall planting season. According to the Ministry of Agriculture, 10,835 dunum of agricultural land has been cultivated with potatoes in the year 2012/2013, and the annual total production of potatoes is estimated at 37,552 tonnes. Accordingly, potatoes constitute 1.3% of total agricultural land cultivated in the West Bank.

Potato is considered as a source of both food and cash income. A recently survey study in the West Bank confirmed that the estimated average income earned by Potato farmer's household from agriculture is the highest income compared with other cultivated crops income. According to the survey results about the monthly consumption of many cultivated crops, potato has the fourth rank among the most consumed field crops after wheat, flour and tomato in the West Bank.



**CHAPTER TWO**  
**LITERATURE REVIEW**

## 2.1 Potato Infecting Viruses.

Potato is susceptible to a wide range of pathogens including bacteria, fungi, nematodes, viruses and viroids (8). It is known to be infected by 175 diseases in addition to several physiological disorders (14). Viruses beside *Phytophthora infestans* (the causative agent of late blight) are the most important ones among potato pathogens (4, 8). Yield reduction of potato is attributed by many of these diseases, for example, the natural infection with potato virus Y could cause a yield reduction up to 80% depending on the virus strain and potato cultivars (12). Apart from late blight, viruses are the most demonstrable seed-borne agents that affect vigour, yield and tuber quality of potato (27, 4, 9). More than 40 viruses naturally infect cultivated potato (Table 1). Some of these viruses notably *Potato leafroll virus* (PLRV), *Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus X* (PVX), *Potato virus M* (PVM) , *Potato virus S* (PVS) and *Potato aucuba mosaic virus* (PAMV) occur worldwide in potato crops, while others are important only in some geographical areas (25, 4, 21, 13,10,8).

In order to spread successfully, Potato viruses need other agent to transfer from infected plants into other healthy ones, in a process called transmission. The transmission of any plant virus needs a cooperation of the virus, the transmitting agent, the plant and the environment under optimum conditions to achieve maximum efficiency (9).

Potato viruses are naturally transmitted via three ways. The first way is through the parts of plants that used for propagation. Vegetative

propagation of infected tubers is considered as the main source of virus infection through which the viruses pass from one generation to the next. Insects as vectors are the second way for virus transmission; these could be mites, aphids, nematodes or fungi. Among the insect vectors of potato viruses aphids are the most important ones, because the two most damaging viruses in the crop, PVY and PLRV, are transmitted via aphid species. Over 50 aphid species are known to transmit potato viruses; they transmit the largest number of viruses (34). Another way for viruses transmission is mechanical transmission, by contact between infected and healthy plant parts, including contact with contaminated farm machinery, operator hands, or animals (8). The viruses which are most frequently encountered in potato fields such as PVY, PVX, PVS, PVA, CMV, PVV and TMV, are mechanically transmitted with the exception of PLRV which is transmitted only by insect vector (10, 25, 46).

Virus infections cause plant disease by affecting their metabolism. In virology plant called primary diseased when they become infected during cultivation, while for plant that originated from infected tubers are secondary diseased plants. Plants with primary and secondary infection with the same virus usually show different symptoms (4). Viruses affect the potato plants in different ways; they cause rapid degeneration of potato tubers, foliar malformation, mild or sever mosaic, stunted growth and wilting. The severity of viruses' symptoms on potato depends on many factors including, potato varieties, virus strain, time of infection, age of crop and environmental conditions (9, 8, 4). Indeed most symptoms of

potato viruses are masked at high temperatures, this makes indexing of virus by visual observation very difficult, and many plants will look healthy while they are actually infected (31). Thus, in order to manage viruses' diseases and control their spread, reliable virus detection methods are needed.

**Table 2.1.1: Distribution and transmission of potato (*Solanum tuberosum*) infecting viruses, adapted from: (31).**

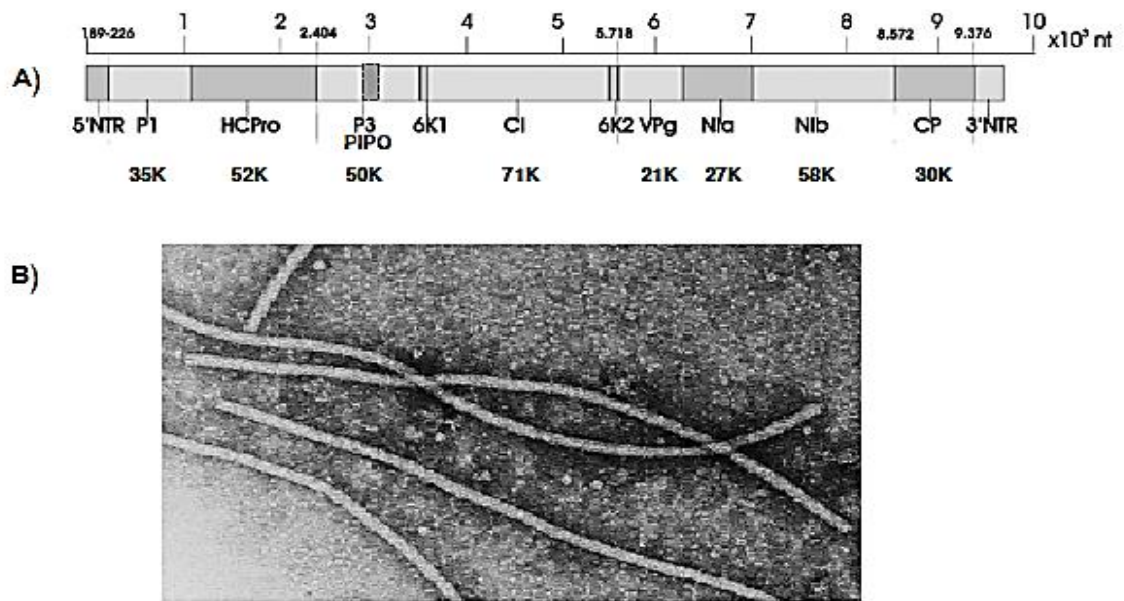
| Virus                                 | Acronym | Virus group                 | Geographical distribution               | Vector        | Mode of transmission/spread |
|---------------------------------------|---------|-----------------------------|---|---------------|-----------------------------|
| Potato virus A                        | PVA     | <i>Potyvirus</i>            | Worldwide                               | Aphids        | Nonpersistent               |
| Potato virus V                        | PVV     | <i>Potyvirus</i>            | Europe and America                      | Aphids        | Nonpersistent               |
| Potato virus Y                        | PVY     | <i>Potyvirus</i>            | Worldwide                               | Aphids        | Nonpersistent               |
| Henbane mosaic virus                  | HeMV    | <i>Potyvirus</i>            | Europe                                  | Aphids        | Nonpersistent               |
| Potato virus X                        | PVX     | <i>Potexvirus</i>           | Worldwide                               | Fungus        | Mechanical contamination    |
| Potato leaf roll virus                | PLRV    | <i>Luteovirus</i>           | Worldwide                               | Aphids        | Persistent                  |
| Potato virus M                        | PVM     | <i>Carlavirus</i>           | Worldwide                               | Aphids        | Nonpersistent               |
| Potato virus S                        | PVS     | <i>Carlavirus</i>           | Worldwide                               | Aphids        | Nonpersistent, contact      |
| Potato virus T                        | PVT     | <i>Capillovirus</i>         | South America                           | None reported | Infected tubers             |
| Andean potato mottle virus            | APMV    | <i>Comovirus</i>            | Peru, Bolivia                           | Beetles       | Contact                     |
| Cucumber mosaic virus                 | CMV     | <i>Cucumovirus</i>          | England, Scotland                       | Aphids        | Nonpersistent               |
| Potato mop-top virus                  | PMTV    | <i>Furovirus</i>            | South America, North and Central Europe | Fungus        | Fungal spores               |
| Alfalfa mosaic virus                  | AMV     | <i>Alfalfa mosaic virus</i> | Worldwide                               | Aphids        | Nonpersistent               |
| Tomato black ring virus               | TBRV    | <i>Nepovirus</i>            | Northern and Central Europe             | Nematodes     | –                           |
| Potato black ring spot virus          | PBRV    | <i>Nepovirus</i>            | Andean region                           | Nematodes     | –                           |
| Tobacco rattle virus                  | TRV     | <i>Tobravirus</i>           | Europe, Americas, and Asia              | Nematodes     | –                           |
| Beet curly top virus                  | BCTV    | <i>Geminivirus</i>          | Utah                                    | Leaf hopper   | Circulative                 |
| <i>Solanum</i> apical leaf curl virus | SALCV   | <i>Geminivirus</i>          | Andes, India                            | Leaf hopper   | Circulative                 |
| Potato yellow dwarf virus             | PYDV    | <i>Rhabdovirus</i>          | Canada, USA                             | Leaf hopper   | Circulative                 |
| Tobacco mosaic virus                  | TMV     | <i>Tobamovirus</i>          | Worldwide                               | Fungus        | Contamination               |
| Tobacco necrosis virus                | TNV     | <i>Necrovirus</i>           | Netherlands, Italy                      | Fungus        | Contamination               |
| Tomato spotted wilt virus             | TSWV    | <i>Necrovirus</i>           | Worldwide                               | Thrips        | Propagative                 |
| Tobacco streak virus                  | TSV     | <i>Illavirus</i>            | Europe                                  | Thrips        | Propagative                 |
| Andean potato latent virus            | APLV    | <i>Tymovirus</i>            | Andean region                           | Beetles       | Contact                     |
| Arracacha virus B                     | AVB     | –                           | Peru                                    | –             | Infected tubers             |
| Egg plant mottled dwarf virus         | EMDV    | <i>Rhabdovirus</i>          | Iran                                    | –             | Infected tubers             |
| Potato aucuba mosaic virus            | PAMV    | –                           | Worldwide                               | –             | Infected tubers             |
| Potato yellow vein virus              | PYVV    | –                           | Andean region                           | –             | Infected tubers             |
| Potato deforming mosaic virus         | PDMV    | –                           | Andean region                           | –             | Infected tubers             |

## 2.2 Potato Virus Y (PVY).

Potato virus Y (PVY) is one of the most damaging species in the genus *Potyvirus*, of the *Potyviridae* family (22). The *Potyvirus* group [ named after its prototypical member, Potato Virus Y], is known as the largest genera of plant viruses, with nearly 150 aphid transmitted viruses including

number of economically important viruses such as PVY, PPV, BYMV and PRSV (23). It is considered as one of the largest plant virus genera that are accounted for 40% of losses caused by all plant viruses (11, 23). PVY occupied the fifth position among the top ten economically damaging plant viruses in the world (13, 29). Together with potato leaf roll virus (PLRV), PVY has been the most significant causes of what used to be called “degeneration” or “running off potato”. It is considered to be the most common virus affecting potato throughout the world (8, 9, 25, 14).

PVY possesses a non-enveloped, filamentous particle, with 730-740 nm long and a diameter of 11-12 nm. It has a single-stranded, positive-sense RNA genome of approximately 10 kb in length, with a viral genome-linked protein (VPg-pro) covalently attached to its 5' end and a poly-A tail present at the 3' end. It was believed that the PVY genome encodes a large- single open reading frame (ORF) which is cleaved by three virus-encoded proteinases into nine functional proteins, (P1, HC-Pro, P3, 6k1, CI, 6k2, NIa, NIb et CP), these viral proteins are involved in different steps of the viral cycle (Figure 2.2.1). The main functions of the PVY encoded proteins are summarized in Table 2.2.1. Recently another short open reading frame which is called PIPO (Pretty Impressive Potyviridae ORF) has been discovered within the Turnip mosaic virus (36, 29). In PVY the PIPO is expressed within the N-terminus of the P3 cistron, therefore it is referred to P3-PIPO, it is known to be involved in cell to cell movement and viral symptom expression within the host plant (36).



**Figure 1.2.1:** A) The genomic organization of PVY; P1, proteinase; HCPro, helper component-proteinase; P3, third protein, 6K1, 6-kDa protein 1; CI, helicase; 6K2, 6-kDa protein 2; V, VPg – viral genome-linked protein; P, Nla– proteinase; CP, coat protein. B) An EM picture for PVY particles.

**Table 2.2.1: Characterization of *Potyviridae* proteins including their function and subcellular localization, adapted from: (32).**

| <b>Protein (size)</b> | <b>Function</b>  | <b>Cellular localization</b>   |
|-----------------------|--|--|
| P1 (32 – 64 K)        | serine proteinase with <i>cis</i> -cleavage activity, P1/HCpro cleavage site. Responsible for symptomatology. Usable for investigation of potyviruses phylogeny and relationship | Crystalline inclusions in cytoplasm and nuclei.  |
| HCpro (56 – 58 K)     | cysteine proteinase with <i>cis</i> -cleavage activity, HCpro/P3 cleavage site. Aphid transmission, systemic movement, suppression of gene silencing, symptoms development.      | Cytoplasm, aggregates along the endoplasmic reticulum.   |
| P3 (37 K)             | plant pathogenicity – virulence, viral infection cycle.  | Crystalline inclusions in cytoplasm and nuclei. Endoplasmic reticulum (punctuate inclusions in association with Golgi) |
| 6K1 (6 K?)            | cell-to-cell movement?, role in virulence?   | The cell periphery.  |
| CI (70 K)             | RNA helicase required for genome replication. Cell-to-cell movement. Long-distance movement. Development of symptoms. Breaking the host resistance.                              | Cytoplasm, conical structures attached to plasmodesmata.   |
| 6K2                   | anchoring the viral replication complex to membranes. Long-distance movement. Development of systemic infection.   | Crystalline inclusions in cytoplasm and nuclei.  |
| VPg                   | covalently links the 5' end of the viral RNA via tyrosine residue. Viral cycle. Systemic infection. Overcoming the eIF4E-based recessive resistance.                             | Endoplasmic reticulum, nucleus, nucleolus.   |
| NIa-Pro               | NIa-pro cysteine proteinase. Host specificity. Host DNA cleavage activity.   | Nuclei of infected cells in the form of inclusion bodies.  |
| NIb                   | RNA-dependent RNA polymerase. Genome/viral replication.  | Nuclei of infected cells in the form of inclusion bodies. Cytoplasm.   |
| CP                    | Aphid transmission. Cell-to-cell and long-distance movement. Virion assembly.  | Plasmodesmatal connections between infected leaf mesophyll cells. Cytoplasm of infected cells.                         |

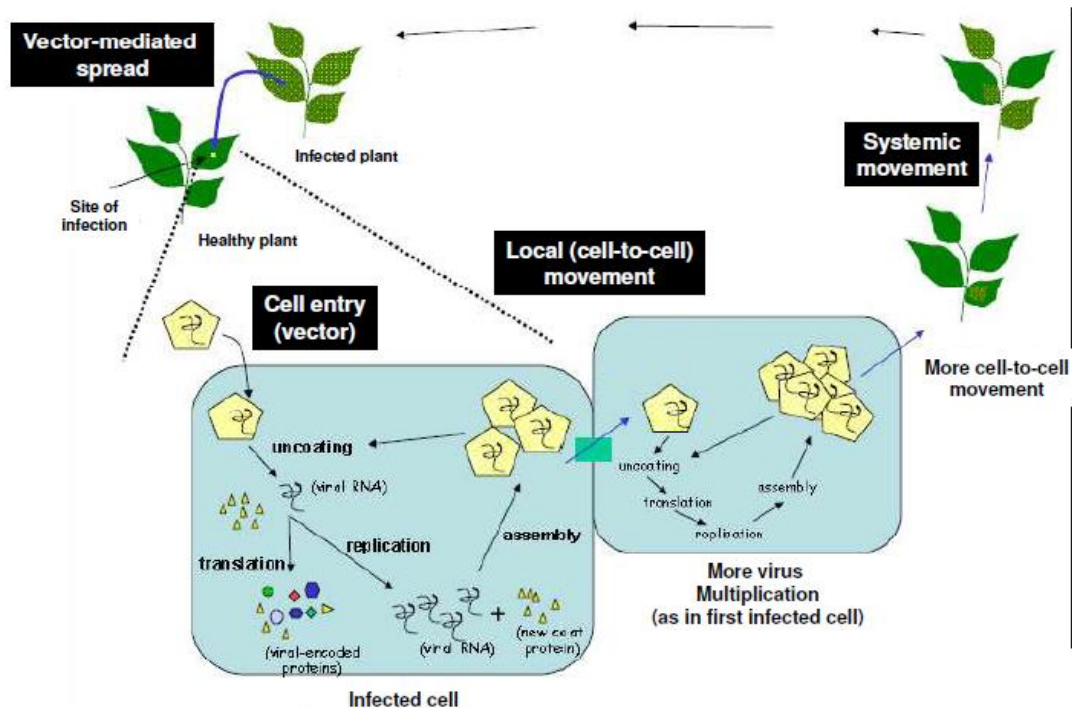
PVY has a worldwide distribution with a broad host range. Apart from potato (46), it is also capable of infecting tobacco, tomato and pepper as well as many solanaceous and non solanaceous weeds (19, 22, 26, 2). The virus is transmitted by aphids in a non-persistent, non-circulative manner, using a helper component protein (HC-Pro). It is known to be transmitted by a wide range of aphid species, in particular the peach potato aphid (*Myzus persicae*) which is considered as the most efficient species

(26,14,16), as well as by mechanical contact, or through infected tubers. In non-persistent transmission, the virus can be acquired within seconds. The virus is lost from the aphid within a few hours after feeding on a new plant, so it is only transmitted for a short distance, up to 100 meters (16,15,19).

PVY and all positive single stranded (+) ss-RNA viruses generally follow the same mechanisms in order to replicate their genome (33). The plant becomes infected with PVY via an aphid vector. Once the virus enters the plant cell through the cytoplasm, its RNA becomes uncoated. The uncoated RNA uses for two purposes, it is used as a messenger RNA (mRNA) which encodes newly viral proteins using the hosts' translational machinery, and it is also act as a template for synthesis of negative stranded RNA. Number of viral proteins such as the NIb and VPg are required for the virus genome replication and the initiation of the viral replication complex (VRC) which contains both viral and host proteins. The RNA-dependent RNA polymerase (RdRp) makes a copy of the positive sense RNA, using the VPg as a primer to create a negative sense copy of the genome, the strands are separated by RNA helicase CI. The negative strand then acts as a template to synthesize more (+) ss-RNA genomes. Newly synthesized RNA strands is either used as templates to generate more RNA strands, or used as mRNA to synthesize viral proteins or can be encapsidated to produce viral particles. The process will continue in a highly regulated manner to ensure large numbers of progeny RNA are produced. The virus multiplication occurs in the leaf cells, and when the virus concentration reach a certain level it is transported via phloem vessels with other



macromolecules to other parts of the plant including newly developed leaves, flowers, roots and tubers in potato (34, 10), see Figure 2.2.2



**Figure 2.2.2: Stages of plant virus life cycle.** The infection of single cell, local cell-to-cell movement, systemic movement and transmission to a different plant (34).

RNA viruses have the highest genome mutation rate among all organisms with an error rate of  $10^{-4}$  to  $10^{-6}$  mutations per base pair per generation, which produces highly evolving viruses due to their huge evolutionary potential, in particular, viruses with positive single stranded RNA genome which characterized by large population sizes, fast replication and high mutation rates (37). The genomic variations mainly exist in two forms; mutation and recombination. Mutations are the most common form in the RNA genome, they mostly occur during genome replication due to the lack of proof-reading activity of the virus-encoded RdRp (replicase) of the newly synthesized RNA strand (47). Moreover, recombination is

considered as the major factor in the evolution of RNA viruses (39). Three models of recombination has been explained, namely the replicase driven template switching model, RNA breakage and ligation model and breakage induced switching model. Recombination is common in potyviruses; also it probably influences the evolution of PVY populations since most of the currently widespread isolates are considered to be recombinant forms (38). The replicase mediated template-switching model is the most common mechanism leads to new RNA virus recombinants (48). This mechanism suggests that recombination between two parental viruses; "donor" and "acceptor" occurs via template switching. During the replication of RNA strand of the donor virus, the polymerase switches the strands at a region of homology between the two viruses resulting in formation of a new virus recombinant (47).

The effect of the PVY on the crop varies depending on the virus strain, host resistance, time of infection and environmental condition. Yield loss is considered to be the most significant effect (17). Many virus related symptoms can be observed within potato crops such as mild to severe mosaic, mottling, chlorosis, necrosis in addition to other symptoms which observed in cases of mixed infection with another virus, as in the combination with *Potato virus X* which causes a disease called 'rugose mosaic'(9). The severity of disease caused by PVY is related to the virus strains. Some newly detected recombinant strains are able to cause serious tuber losses in some potato cultivars (20). This highlights the importance of

identifying which strains are existing in the fields and what their related effects on the local cultivated varieties in that region.

PVY is a rapidly evolving virus which exists as a complex of strains (49, 50). The virus was originally distinguished according to symptomatology, which is considered as traditional method for virus identification depending on the biological properties of the virus. Using this approach PVY were generally classified into three major groups: the ordinary (PVY<sup>O</sup>), the stipple streak strain (PVY<sup>C</sup>), and tobacco vein necrosis (PVY<sup>N</sup>) (3, 38). Currently, seven strains of PVY have been identified based on host response and resistance gene interactions (51, 35). Five distinct strain groups are defined that cause local or systemic hypersensitive response (HR: form a resistance characterized by a localized cell death at infection sites) in genetic background with a corresponding N gene: PVY<sup>O</sup>, PVY<sup>N</sup>, PVY<sup>C</sup>, PVY<sup>Z</sup>, PVY<sup>E</sup> in addition to PVY<sup>NTN</sup> and PVY<sup>N-Wi</sup> which have been identified based on molecular characterization (3, 51).

PVY<sup>O</sup>, the oldest known form of virus Y which was first identified by Smith (1931). This virus strain group is found worldwide. In general it causes severe symptoms such as crinkling, rugosity or leaf-drop streaks and stunting although the type and severity of symptoms may depends on potato genotypes. This strain does not cause vein necrosis in tobacco; it usually induces symptoms ranging from typical mild mosaic to systemic mottle (9, 17). Moreover it causes mosaic symptoms when it became inoculated into tobacco plants (52).

PVY<sup>C</sup>, the stipple streak group. Was identified in the 1930s in Netherlands (8, 19). This strain causes mosaic patterns or stipple streak in susceptible potato and identified by an HR response to Nc resistance gene. Symptoms in tobacco are reported to be indistinguishable from those induced by PVY<sup>O</sup> isolates. Unlike the other strains of PVY, some PVY<sup>C</sup> strains are non-aphid transmissible (17, 8, 52).

PVY<sup>N</sup>, stands for new virus Y or (necrotic virus Y) that was detected In the 1950s in many countries in Europe as a new variant of PVY. It causes veinal necrosis and has the potential to producing yield losses of 100% in tobacco. It is frequently undetected in potato or could produce mild mosaic in foliage (18, 8). This strain group has been known of its ability to overcome the resistance genes (Ny, Nc and Nz) which elicit hypersensitive responses in cultivars (3). PVY<sup>E</sup>, is mimicking the PVY<sup>N</sup> strain in its ability to overcome HR responses in potato, but it doesn't produce vein necrosis in tobacco as the PVY<sup>N</sup> (51).

PVY<sup>Z</sup>, was identified in 1980s and characterized by its ability to overcome both Ny and Nc resistance genes, while eliciting HR in some potato cultivars known to harbor the Nz resistance gene. Significance and geographical distribution of both PVY<sup>E</sup> and PVY<sup>N</sup> strain groups has never been reported (3, 52).

The strain PVY<sup>NTN</sup> was first reported in Hungary in 1980, and characterized at molecular level as a recombinant strain group of PVY. Since then outbreaks have been reported in many regions of the world. In

addition to the primary symptoms in the foliage as it causes severe mosaic, this strain is able to produce potato tuber necrotic ringspot disease (PTNRD) which affect the tubers in a form of necrotic rings makes them unmarketable (8, 17). Thus it is called the NTN because it had the same biological and serological features of PVY<sup>N</sup> while the TN stands for tuber necrosis (34).

PVY<sup>N-Wilga</sup> or PVY<sup>N:O</sup> was first identified in 1991 in Europe. This recombinant strain group is characterized by its symptomless infection (53). Therefore it is difficult to detect it in the field due to the lack of symptoms it produces in the plant. In some cultivars it produces a systemic mottle and dwarfing, but it does not cause necrosis in the tubers. PTNRD initiation has been reported with this strain (54). In addition 90% of PVY isolates which surveyed in Poland belong to the PVY<sup>N-Wilga</sup> group and cause significant yield losses in potato crops (55).

### **2.3 Detection Methods of Plant Viruses.**

Viruses infect a wide range of plant species leading to cause many serious plant diseases in the world. The effect of viral diseases on plants quality and quantity leads to the loss of billions of dollars per year (56, 57). Unfortunately, there are no chemical agents like bactericides and fungicides that can use against plant viruses (22). Viral diagnosis is considered as one of the most valuable strategies for virus disease management. Thus to achieve an effective control, viruses must be accurately identified as an initial step of management. Diagnostic and

detection techniques for plant viruses categorize based on: biological properties of the virus interaction with its host or vector, such as symptomatology and transmission tests and intrinsic properties of the virus itself, like serological and nucleic acid-based techniques (22, 57).

### **2.3.1 Diagnostic methods based on biological properties.**

#### **i. Symptomatology.**

Symptoms on plant usually used if they are characteristic of a certain disease (47). Symptomatology relies on visual inspection of the symptoms expressed by viruses in plant. Plant viruses cause many symptoms on plant like mosaic, mottling, stunting, leaves malformation, necrosis, chlorosis, yield reduction or a combination of these symptoms. However, many biotic and abiotic factors could affect the appearance of these symptoms. Moreover, some plants could exhibit virus-like symptoms as a result of unfavourable conditions. Although symptoms provide essential information about virus infection, we can't make a decision on symptomatology alone, as some viruses cause symptomless infection in plant. This method is usually done in conjunction with other confirmatory tests to achieve the maximum accuracy of viral diagnosis (24).

#### **ii. Biological assay (Transmission tests).**

It is the one of the oldest methods for plant viruses' diagnosis. It is a traditional method that still uses in many laboratories as an important assay for virus detection and identification (47). It includes mechanical, graft, and

vector transmission of the virus to susceptible herbaceous indicator plants. Mechanical transmission using sap inoculation to indicator plants can be done easily with minimum facilities. The characterizations of symptoms produced on these plants allow detection and identification of many viruses. Viruses that are not able to be transmitted via mechanical transmission as well as viruses of tree fruit can be diagnosed via vectors or grafting using suitable indicator plants. Although these assays are usually used for routine diagnosis of viruses, they may provide imprecise virus identification in addition they consume time and resources (24).

### **iii. Microscopy.**

Electron Microscopy (EM) provides useful information about virus morphology. For stable viruses as well as viruses with filamentous and rod-shaped particles, rapid results can be observed using negative staining technique. Even though, it is not easy to detect viruses with isometric shape. Moreover virus particles that occur in low concentration, need to be concentrated in plant sap or captured using antibody-coated grids (Immunosorbent Electron Microscopy) to achieve high efficiency of virus visualization. EM is commonly used for virus detection when the needed facilities are readily available, thus it cannot be used for rapid detection of multiple samples as it is very expensive and labor intensive method. Many plant viruses such as potyviruses form cylindrical inclusion bodies in infected cells. They develop large crystalline accumulations of virus particles, thus make their detection by EM or light microscopy a simple,

rapid, and relatively inexpensive method to confirm viral infection. This cylindrical inclusions (CI) bodies are formed by a virus-encoded protein and can be considered as the most important phenotypic criterion for assigning viruses to certain genus level using selective stains (23, 24).

### **2.3.2 Serological Methods.**

Serology is one of the most easiest and specific methods for virus diagnosis that gives a rapid and precise outputs. All of serological assays depend on the virus coat protein properties and fall into two types, solid phase assays (ELISA, western immuno-blotting) and liquid phase assays (precipitation and agglutination tests). Serological techniques which include enzyme-linked immunosorbent assay (ELISA) and tissue immuno-blotting assay considered as powerful tools for plant viruses detection. These techniques are based on an antigen-antibody reaction between virus specific coat proteins (epitopes) and anti-viral antibodies that raised in mammalian systems like rabbits and mice, therefore it can be visualized using several detection means such as enzyme-labelled antibodies (58).

#### **i. Enzyme-linked immunosorbent assay (ELISA).**

Enzyme-linked immunosorbent assay is the most significant and popular advance among serological tests, especially in virus detection (60). In 1977 ELISA was introduced to plant virology by Clark and Adams. Since then it has been very common for plant virus detection in plant material, insect vectors, seeds and vegetative propagules (61). ELISA is widely used for the



detection of plant viruses due to its simplicity, adaptability, reliability, sensitivity in addition to its economy, thus it is used to test large number of samples in a relatively short period of time (45, 24) . The basic principle of the ELISA based on immobilizing the antigen onto a solid surface, or trapping antigen by specific antibodies, and probing with specific immunoglobulins attached to an enzyme label. The positive reaction is detected by adding the suitable substrate. The enzyme converts substrate to product, which can be easily visualized due to its color. Many variations of ELISA have been developed (Figure 2.3.2.1). These variations are categorized into, "direct" and "indirect" forms of ELISA. Both categories have the same underlying theory and the same final results, meanwhile they differ in the way of detection for the antigen-antibody complex. See Figure 2.3.2.2. The disadvantages of ELISA are the time of extraction for plant tissue which can take several hours as well as the incubation times required for samples and antibodies in order to be adhered into microtiter wells (24, 59).

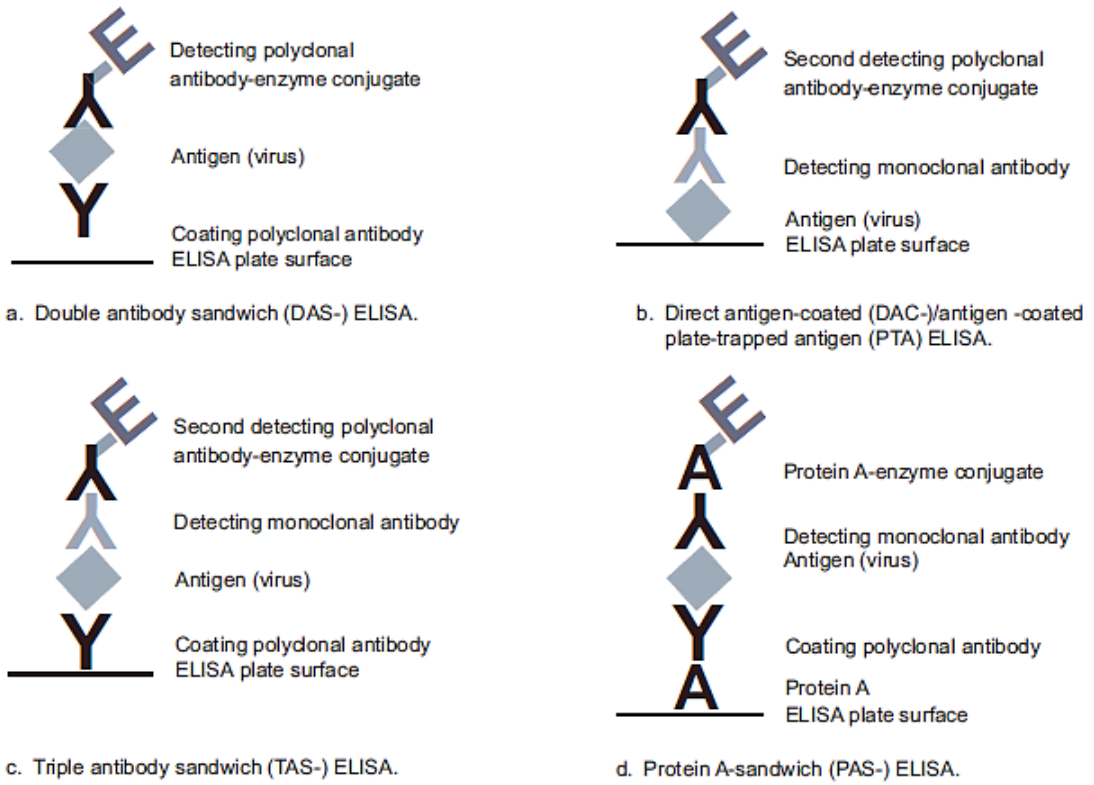
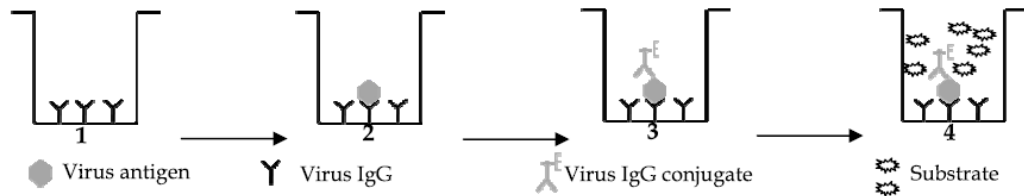


Figure 2,3.2.1: The different variations of enzyme-linked immunosorbent assay (24).

**A- Direct ELISA - DAS-ELISA**



**B- Indirect ELISA - PTA-ELISA**

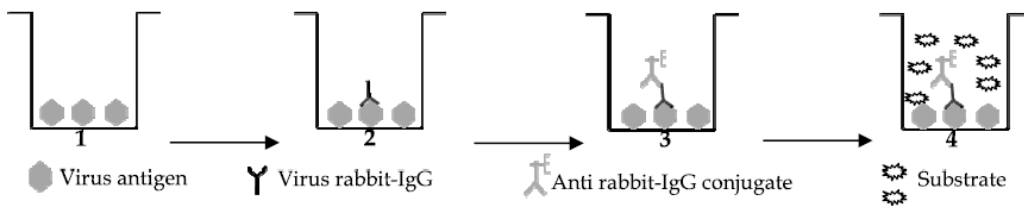


Figure 2.3.2.2: The two categories of ELISA: A- Direct ELISA and B- Indirect ELISA . The drawings illustrate the most frequently used methods for detection of plant viruses (59).

## **ii. Tissue blotting immunosorbent assay (TBIA).**

TBIA is a variation of dot-blot immuno assay (DBIA) which is used for virus detection in both plants and vectors. It was first used in 1990 (63). Since then it became widely used as simple and reliable method for virus detection (62). TBIA has relatively the same technique as ELISA except that a freshly-cut edge of plant or vector is used and spotted onto a membrane instead of using a microtiter plate. Moreover, in ELISA a soluble substrate is used for color development, while a precipitating one is usually used for virus detection in TBIA (64). Although TBIA has some advantages over ELISA, as there is no need for tissue extraction and membranes can be blotted directly in the field and in addition to be processed at a later date, TBIA has several disadvantage is that the results are qualitative, rather than being quantitative as well as it is difficult to observe weak positive reactions due to the interference of sap components with the subsequent diagnostic reaction (65, 24).

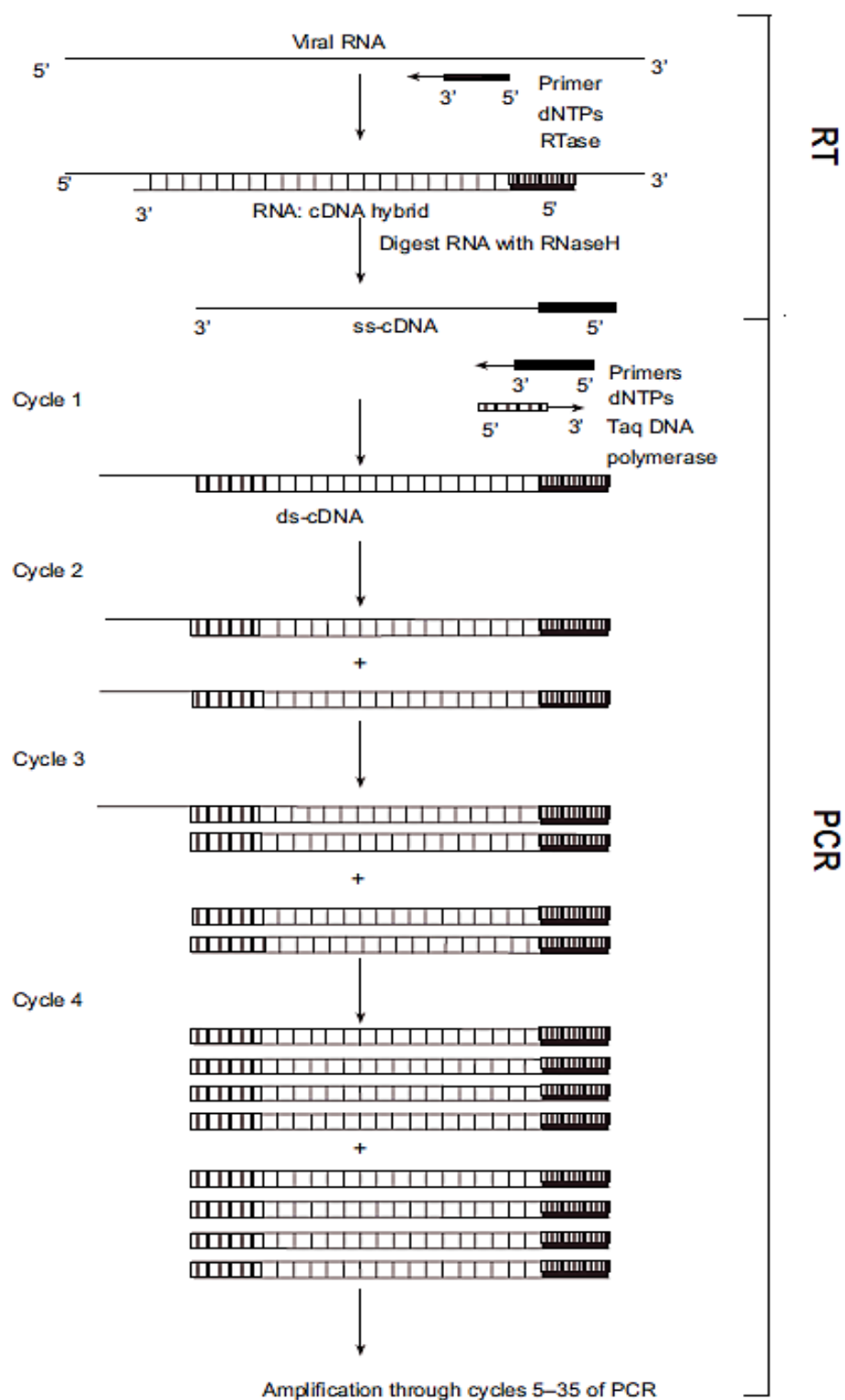
### **2.3.3 Nucleic Acid-based Methods.**

Nucleic acid-based techniques are used extensively for plant viruses' detection and identification, mainly after the advent of the polymerase chain reaction (PCR) (67). Although serological techniques are widely used for virus detection, they have certain drawbacks if compared with nucleic acid-based techniques. They are based on the viral coat proteins and its antigenic properties, which represents only about 10% of the total virus genome (68) thus they neglect the rest of the virus genome. While in

nucleic acid-based detection methods any region in the virus genome can be targeted and diagnosed. Moreover, in some cases serological procedures cannot be used particularly for the detection of viroids, satellite RNAs, viruses which lack particles (e.g., *Groundnut rosette virus* (GRV)), viruses which occur as extremely diverse serotypes (e.g., Indian and African *Peanut clump virus* and *Tobacco rattle virus*) and viruses that are difficult to purify (24).

Polymerase chain reaction (PCR) is an extremely specific, sensitive and versatile in vitro method that have a great potential to amplify trace amounts of targeted nucleic acid using specific primers to the region of amplification, and thermostable DNA polymerase (68, 60). It consists of three step cycles: denaturation in which the two complementary strands of the double-stranded DNA are separated at high temperature (usually 94-95 °C), annealing of two oligonucleotide primers to their complementary sequences in the opposite strands of the target DNA (annealing temperature depends on the nucleotide composition and length of the primer, usually 35-65 °C), and elongation or extension of each primer through the target region (usually at 72 °C) using a thermo-stable DNA polymerase (e.g., Taq polymerase). Each DNA strand made in one cycle will use as a template for synthesis of a new DNA strand in the next cycle. The procedure is repeated many times (30-40 cycles) by an automated thermal cycler until sufficient product of amplicon is produced. This procedure is directly applied for amplification of plant viruses with DNA genomes(eg. Caulimo, Gemini and Badnaviruses) using gene-specific primers to the region of

amplification. While in plant viruses with RNA genomes, the targeted RNA must be converted to a complimentary DNA (cDNA) using reverse transcription (RT) prior the beginning of PCR procedure. See Figure 2.3.3.1. In addition to its usefulness as a diagnostic technique, PCR is used by many research laboratories in the world for many purposes including, molecular characterization (69), DNA comparisons between related pathogen species (70) as well as in evolutionary studies (71). Although PCR can achieve a very high sensitivity and specificity, different comparative assays have been reported a failure of PCR amplification to correctly diagnose infected and non-infected plant material. Although PCR can achieve a high sensitivity and specificity, different comparative assays a failure of PCR amplification to correctly diagnose infected and non-infected plant material has been reported. This failure could be a consequence of the "carry-over" contamination of amplicons that can be responsible for false-positive results and inhibitor components in sample extracts which is the main reason for false negatives (66, 60, 24).



**Figure 2.3.3.1: Diagrammatic representation of reverse transcription-polymerase chain reaction (RT-PCR).** Each cycle of PCR consists of denaturation, annealing, and extension. RTse = reverse transcriptase, ss-cDNA = single-stranded cDNA, ds-cDNA = double-stranded cDNA, dNTPs = deoxynucleotide triphosphates (24).

## 2.4 Objectives.

Within the main scope of revealing the knowledge about the sanitary status of plants and presence of phytoviruses in the State of Palestine, this study was aimed to:

- 1) Establish the status of *Potato virus Y* in potato plants using different detection methods.
- 2) Determine the incidence and distribution of *Potato virus Y* in the main growing regions of potato in the West Bank.

Besides, this study will provide a platform for plant viruses diagnostic research and will be helpful to create proper management approach to control plant viral diseases in Palestine.

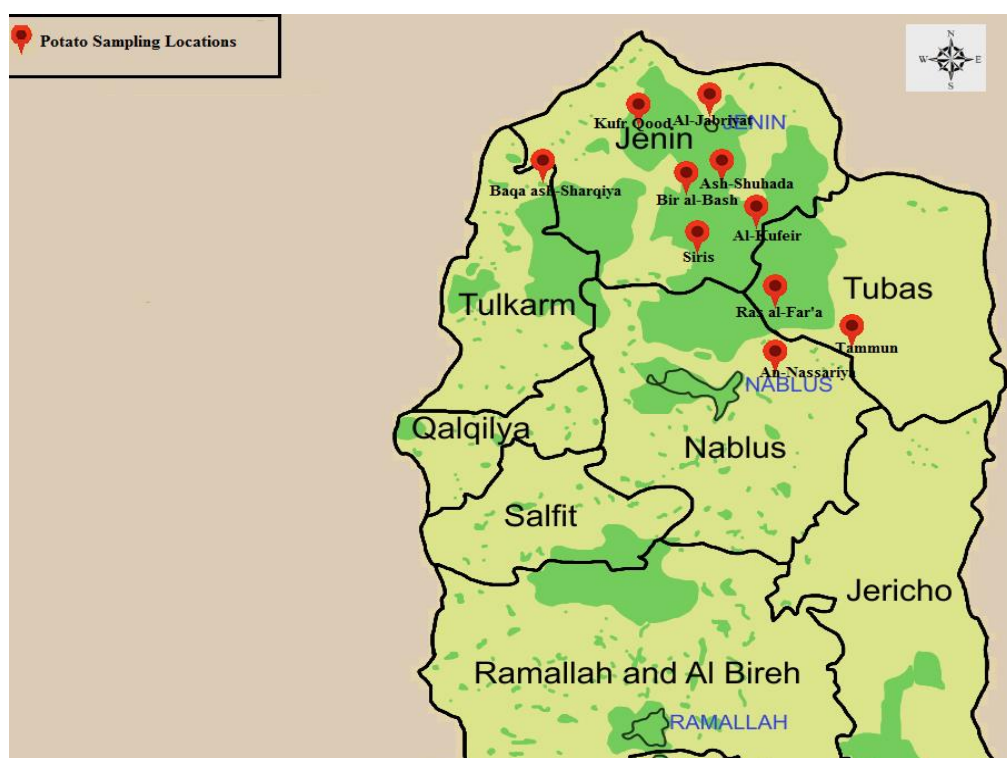
**CHAPTER THREE**  
**MATERIALS AND METHODS**



### 3.1. Samples Collection and Field Survey.

#### 3.1.1. Sampling Locations.

Forty potato fields were selected from 10 locations situated in four districts of West Bank (Jenin, Nablus, Tubas and Tulkarem) (figure 3.1.1.1). The specific locations were Al-Jabriat, Kufr Qood, Bir al-Basha, Ash-Shuhada, Siris, Al-Kufeir, Tammun, Al-Far'a, An-Nasariyah and Baqa ash-Sharqeah. The fields ranged between 2-33 dunum in size and contained different potato varieties.



**Figure 3.1.1.1:** A map shows the sampling locations at each district included in the study.

#### 3.1.2. Sampling in the Field.

A number of 255 potato leaf samples representing eight varieties namely, Spunta, Mondial, Arizona, Desiree, Hermes, Zafira, Faluka and Manitou

were collected as shown in Table 3.2.1.1 . All of samples were collected during the spring growing season of 2015 except samples of Nablus district were belong to the fall season of 2014. The selected potato crops were ranging from 1-5 months in age. The names and ages of potato varieties were recorded as identified by the farmers. According to the owners of the surveyed fields, cultivated potato seeds (tubers) were imported via three main companies: Medraj Company (Tubas), Sharabati Company (Hebron) and Roots Agri. and Trade Company (Tulkarem). In few cases, some famers import the seeds directly from Netherlands via the Ministry of Agriculture.

Using clean gloves, young top leaves were sampled and stored in plastic bags. Potato leaves in the sample bags were immediately stored in a cool box and later transferred to the cold room (4 °C) upon arrival to An-Najah National University Research Lab and used for serological analysis.

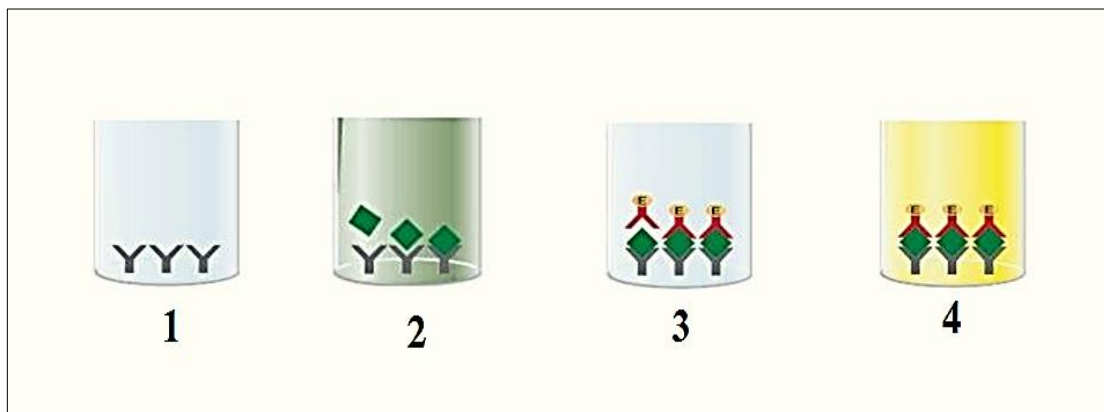
**Table 3.1.2.1: Numbers and distribution of the collected samples according to the potato variety.**

| Potato Varieties | # of samples at each region |       |          |       | Total # |
|------------------|-----------------------------|-------|----------|-------|---------|
|                  | Nablus                      | Jenin | Tulkarem | Tubas |         |
| Spunta           | 50                          | 79    | 4        | 48    | 181     |
| Arizona          | -                           | 24    | -        | -     | 24      |
| Mondial          | -                           | -     | 8        | -     | 8       |
| Hermis           | -                           | 9     | -        | -     | 9       |
| Zafira           | -                           | 10    | -        | -     | 10      |
| Desiree          | -                           | 10    | -        | -     | 10      |
| Manitou          | -                           | 10    | -        | -     | 10      |
| Faluka           | -                           | -     | 3        | -     | 3       |

### **3.2. Serological Detection (ELISA Test).**

The double antibody sandwiched enzyme-linked immunosorbent assay (DAS-ELISA) was conducted using PVY monoclonal cocktail kit from BIOREBA, Art. No: 112975, see figure 3.2.1. Coating monoclonal antibody (MAb) was diluted with coating buffer at 1:1000 dilution, dispensed (200  $\mu$ l) into the wells of an ELISA plate (certified Nunc-Immuno Plates MaxiSorp F96), covered tightly and placed in a humid box, then incubated for 4 hours at 30 °C. The washing buffer, extraction buffer and coating buffer were prepared according to the manufacturer's instructions. The ELISA plates were washed 3-4 times with washing buffer and blotted on paper towels. While incubating the ELISA plates, potato leaf samples were weighed. Extraction buffers (3-4 ml) were added per 0.5 g of sample and grounded using pestle and mortar. 200  $\mu$ l of plant extraction were added into each well. The plate was covered tightly, placed in a humid box and incubated overnight at 4°C. ELISA plates were then washed 3-4 times with washing buffer and blotted dry. Enzyme conjugate was diluted at 1:1000 in conjugate buffer and loaded into test wells (200  $\mu$ l), incubated for 5 hours at 30°C. The ELISA plate was washed and blotted dry. The freshly prepared para-nitrophenyl-phosphate substrate (pNPP) was dissolved at 1 mg/ml in substrate buffer, then added (200  $\mu$ l) into test wells and incubated at room temperature (20-25°C) in the dark. The ELISA test was carried out with several replicates; samples were duplicated including positive and negative controls. ELISA results were recorded visually (color

changing) and photometrically using ELISA reader with dual filters at 405/492 nm.



**Figure 3.2.1: DAS-ELISA steps.** 1- Coating: PVY monoclonal antibody were adsorbed to surface of microtiter wells. 2- Antigen: Incubation of plant extract. 3- Conjugate: Incubation of enzyme-labeled antibody. 4- Substrate: Color reaction indicates infected sample.

### 3.3. Biological Assay (Mechanical Inoculation).

Certified seeds of different indicator plants *Nicotiana benthamiana* and *Nicotiana tabacum* were planted in sterilized soil. Infected leaf tissues were ground using a sterile mortar and pestle after the addition of ~3-4 ml of pH7 0.1 M phosphate buffer to make leaf sap (inoculum). Celite powder was previously dusted onto leaves of indicator plants, Inocula were applied uniformly over indicator plant leaves with index finger, by starting each stroke from the petiole, and ending at the leaf tip (Figure 3.3.1). The indicator plants were inoculated with plant mixture extracted from uninfected plants and used as control set.



**Figure 3.3.1:** Mechanical inoculation of PVY.

### 3.4. Incidence Study.

The incidence of PVY infection was determined for each potato growing region. In each field, 4-6 rows were chosen in a random manner, and samples number for each field was taken according to field size.

In order to detect the prevalence of PVY, all samples were tested against PVY using direct ELISA, as mentioned before in the material and methods (3.2). The percentage (%) incidence of PVY was calculated by following formula (40).

$$\% \text{ Incidence PVY} = \frac{\text{Potato samples confirmed positive by ELISA}}{\text{Total potato samples tested}} \times 100\%$$

### 3.5. Molecular Detection.

#### 3.5.a. Total RNA Extraction.

Total RNAs were extracted using PureLink® RNA Mini Kit (Life technology™, Catalog No. 12183018A). Liquid nitrogen was added to a sterile mortar then frozen tissue (potato leaves) were grinded thoroughly

using a sterile pestle. The tissue powder transferred to an RNase-free, appropriately sized round-bottom microcentrifuge tube that had been cooled on ice. Immediately the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol were added to each sample (1.5 ml per 0.25 g tissue powder). The lysate was homogenized using vortex in order to disperse the sample, and then it was incubated for 3 minutes at room temperature.

A volume of 350  $\mu$ l lysate was transferred into a clean homogenization tube, and Centrifuged at 13,000 rpm for 5 minutes. One volume 70% ethanol was added to each volume of the homogenate. Samples were mixed to mix thoroughly using vortex to disperse any visible precipitate that may form after the addition of ethanol. Up to 700  $\mu$ l of the sample were transferred (including any remaining precipitate) to a spin cartridge (with a collection tube). Samples were centrifuged at 13,000 rpm for 15 seconds at room temperature, then the flow-through was discarded, and the spin cartridge reinserted into the same collection tube. After that a 700  $\mu$ L Wash Buffer I were added to the spin cartridge. Again samples were centrifuged as the previous step, but the spin cartridge inserted into a new collection tube. 500  $\mu$ l of Washing Buffer II with ethanol were added to the spin cartridge and centrifuged at 13,000 rpm for 15 seconds at room temperature, and then flow-through was discarded. The last previous steps were repeated once. The spin cartridge centrifuged at 13,000 rpm for 1–2 minutes to dry the membrane with bound RNA. The collection tube was discarded and the spin cartridge inserted into a recovery tube. 50  $\mu$ l of

RNase-free water were added to the center of the spin cartridge and incubated at room temperature for 1 minute, then the spin cartridge centrifuged for 2 minutes at  $\geq 13,000$  rpm at room temperature to elute the RNA from the membrane into the recovery tube. Finally purified RNA was stored at  $-20$  °C.

### **3.5.b. Reverse Transcription (First Strand cDNA) Synthesis.**

Total RNA was used for complementary DNA (cDNA) synthesis. The cDNA was synthesized in a 20  $\mu$ l reaction using M-MLV reverse transcriptase. A volume of 10  $\mu$ l purified RNA was mixed with 1  $\mu$ l of the initiation primer M4T (5'-GTT TTC CCA GTC ACG AC (T)<sub>15</sub>-3') (41, 42), denatured at 95° C for 5 min. then quickly chilled on ice. Reverse transcription was done for 2 h. at 37° C by adding:

- 4  $\mu$ L of M-MLV buffer 5 X,
- 0.5  $\mu$ L of 10 mM dNTPs,
- 2  $\mu$ L of 10 mM DTT,
- 1  $\mu$ L of reverse transcriptase ( M-MLV, 200 U /  $\mu$ l),
- 1.5  $\mu$ L of ddH<sub>2</sub>O (RNase free water).

The total cDNA was used immediately for PCR.

**3.5.c. RT- PCR.**

PCR was used to amplify the 3`terminal genomic region of the virus using degenerate primers (Sprimer: 5`-GGX AAY AAY AGY GGX CAZ CC-3`, X = A, G,C or T; Y = T or C; Z = A or G and M4: 5`-GTT TTC CCA GTC ACG AC-3`).The PCR master mix contained 2.5 µl cDNA template, 2 µl of each primer, 0.5 µl of 10 mM dNTP, 10 µl of 10X Taq polymerase buffer, 0.2 µl Taq DNA polymerase (5U/µl), 1.5 µl of 25 mM MgCl<sub>2</sub> and 31.5 µl ddH<sub>2</sub>O.The initial denaturation was at 94°C for 2 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 47°C for 60 s and extension at 72°C for 2 minute. The final extension was carried out at 72°C for 10 minutes (41, 42).

The PCR products were separated by electrophoresis on 1.2% agarose gel in 1x TAE buffer stained with 0.75 µl GelRed (Biotium, USA).



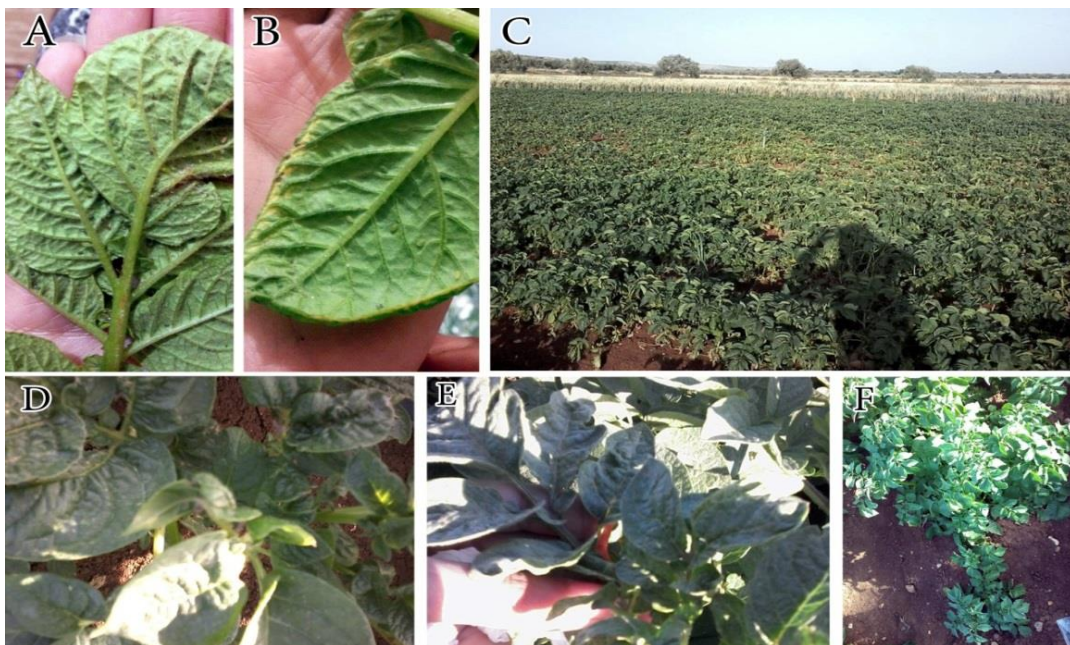
# **CHAPTER FOUR**

## **RESULTS**

#### 4.1. Field Survey Results.

A variety of virus symptoms were observed in the surveyed fields (Figure 4.1.1). In some fields farmers were mixing a number of varieties randomly, as a result, the symptoms were observed regardless the potato varieties. Observed symptoms included yellowish-green mosaic, leaf malformation, stunting, rugosity, yellowing and wilting, in addition to the observation of the main vector of PVY (*Myzus persicae*).

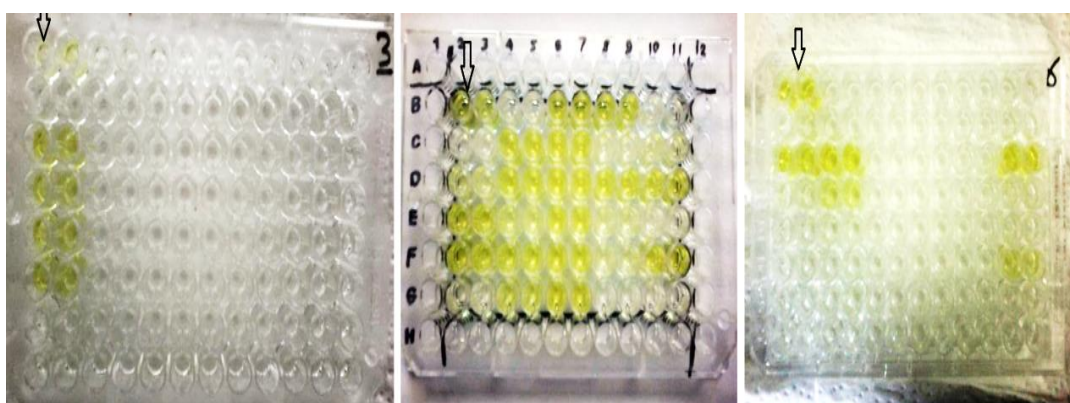
The surveyed fields were planted variably with eight potato varieties as mentioned before (3.1.2). Spunta was the most abundant variety, especially in Nablus district. While some varieties such as Falucka was planted for the first time in Tulkarem surveyed fields, this variety was the least abundant among the others, as seen in Table 4.1.1



**Figure 4.1.1: PVY Observed symptoms during the field survey.** Leaf malformation (A). Yellowing and wilting (B and C). Leaf rugosity (D and E). Plant stunting (F). Yellowish-green mosaic (not shown).

## 4.2. Serological Detection Using DAS-ELISA.

Using DAS-ELISA PVY has been detected in 39 out of 255 samples. Many of these samples were collected from symptomless potato plants. Positive results of DAS-ELISA were judged based on the readings of the ELISA reader compared with the cut off value supplied by the data sheet of the kit, in addition to the development of yellow color in the wells of ELISA plates (Figure 4.2.1).

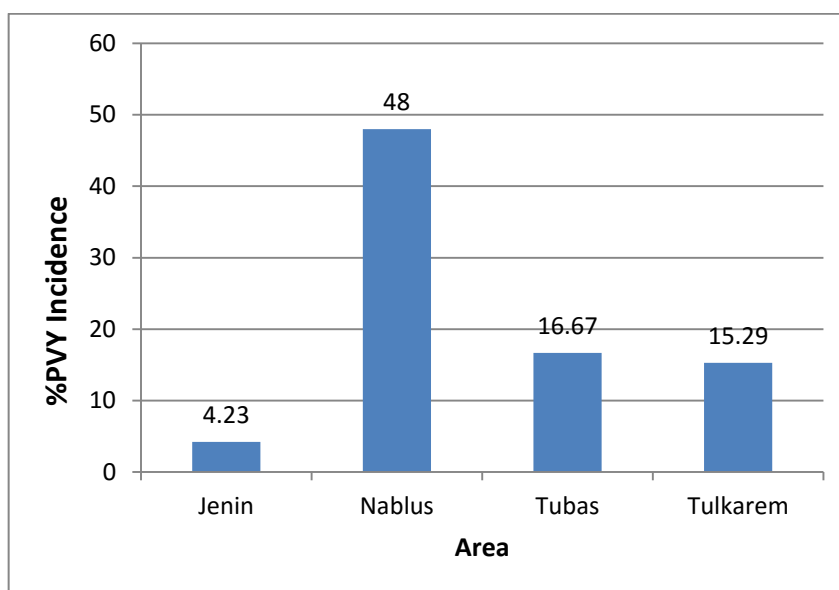


**Figure 4.2.1: DAS-ELISA for detection of PVY.** The yellow color in ELISA plates indicate +ve PVY samples. All samples were tested in duplicates. The positive controls are indicated by arrow in the ELISA plates.

## 4.3. Incidence of PVY.

The incidence of PVY infecting potato was studied in potato main growing regions during the growing seasons of 2014/2015. Maximum PVY incidence was observed at the selected fields of Nablus district which reached 48 %, compared with 16.67 %, 6.67 % and 4.23% for fields in Tubas, Tulkarem and Jenin, respectively (Figure 4.3.1). Of all infected samples that collected from potato fields, 15.29 % were found infected by PVY. Although the virus was detected in all districts from which samples

were collected, some locations were PVY free, see Table 4.3.1. The most infected variety was the Spunta since all of samples that detected as PVY positive were identified as Spunta variety except on sample was belong to the Mondial.



**Figure 4.3.1:** PVY incidence in the surveyed districts.

**Table 4.3.1: The incidence of PVY in each surveyed location.**

| Location        | # of collected samples | # of infected samples | PVY Incidence |
|-----------------|------------------------|-----------------------|---------------|
| Al-Kuffeir      | 58                     | -                     | -             |
| Siris           | 39                     | 2                     | 5.13 %        |
| Ash-Shuhada     | 16                     | 4                     | 25 %          |
| Bir al-Basha    | 19                     | -                     | -             |
| Kufr Qood       | 5                      | -                     | -             |
| Al-Jabriyat     | 5                      | -                     | -             |
| An-Nassariya    | 50                     | 24                    | 48 %          |
| Tammun          | 34                     | 3                     | 8.82 %        |
| Al-Far'a        | 14                     | 5                     | 35.71 %       |
| Baqa ash-Sharqi | 15                     | 1                     | 6.67 %        |

**Chi-Square = 146.367, P-Value = 0.000**

#### 4.4. Mechanical Inoculation.

Mechanical transmission was carried out with a number of ELISA highly positive samples, on herbaceous indicator plants; *Nicotiana benthamiana* and *Nicotiana tabacum*. PVY symptoms appeared after nearly four weeks of inoculation. *N. tabacum* plants that inoculated with PVY showed mild to severe mosaic (figure 4.4.1). In contrast, *N. benthamiana* showed no symptoms.

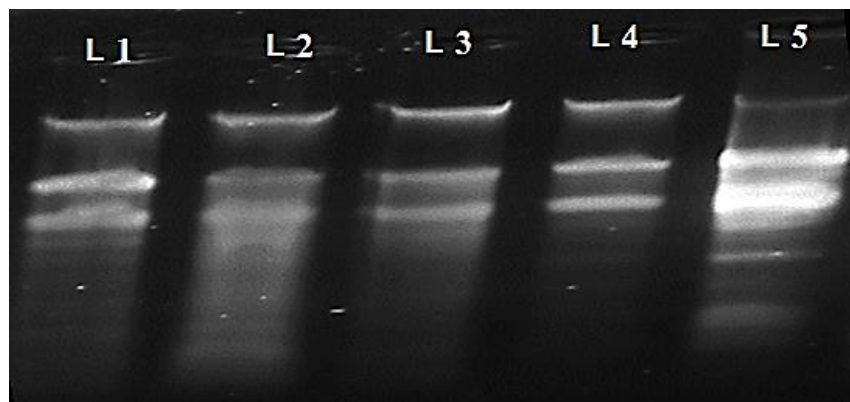


**Figure 4.4.1:** PVY symptoms appeared after four weeks of inoculation on *N. tabacum* plants showed severe mosaic .

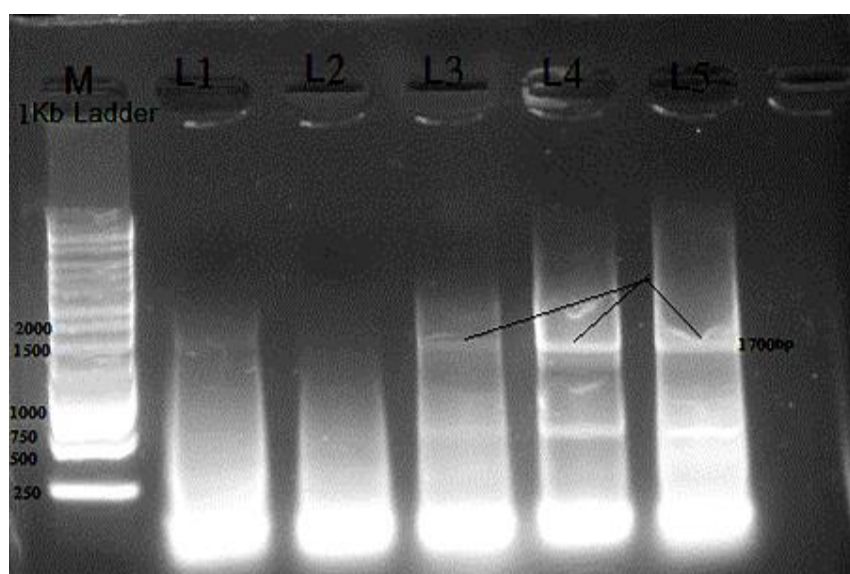
#### 4.5. Molecular Detection Using Degenerate Primers.

Numbers of serologically tested samples were tested at molecular level using universal primers as mentioned before (3.5.c.1). The tested samples were selected based on their positive reactions in DAS-ELISA. Total RNA was successfully extracted from 5 out of 10 samples (Figure 4.5.1). PVY was detected in 3 out of 5 samples with an observed PCR product size of

approximately 1.7 kb on a 1.2 % agarose gel following electrophoresis (Figure 4.5.2).



**Figure 4.5.1:** 1.2% agarose gel illustrate total RNA extraction from 5 different potato leaf samples. Lanes 1-5 represent the total RNA for samples screened with PVY using DAS-ELISA.



**Figure 4.5.2:** RT-PCR detection of PVY 3' terminal genomic region (-1700 bp). Lane M represents 1Kb DNA ladder RTU, (GeneDireX); lanes : 3, 4, 5 represent samples positive with PVY.

**CHAPTER FIVE**  
**DISCUSSION, CONCLUSION and**  
**RECOMINDATIONS**



## **5.1 Discussion.**

Potato is considered as one of the most important crops worldwide as well as in Palestine.

The incidence of Potato virus Y was analyzed in 255 potato leaf samples. Variety of virus related symptoms (yellowish-green mosaic, leaves malformation, stunted growth, rugosity, plant yellowing and wilting) were observed in the surveyed potato fields (43, 44). Although visual inspection is considered as a traditional method for identifying plant viruses in the field, many of asymptomatic plants were screened as PVY positive plants. Consequently this method is insufficient and not always reliable because the development of symptoms usually depends on several factors such as time of infection, plant variety, growth stage, virus strain and other environmental factors (9, 45). In addition some PVY strains can cause symptomless infection in potato plant (18, 8, 53). Moreover, not all plants that showed virus-like symptoms tested virus positive with DAS-ELISA, this might be due to the fact that some plants could exhibit virus-like symptoms as a result of unfavourable conditions. It is also possible that they were infected by other types of plant viruses which cause the same effect on potatoes (24).

All samples were screened using DAS-ELISA technique. This technique successfully detected the virus in potato leaf samples even in the leaves that were asymptomatic. These illustrates were in agreement with previous



findings that DAS-ELISA was a reliable and sensitive method for primary screening of plant viruses (60, 72).

Mechanical inoculation for a number of ELISA highly positive samples was conducted to confirm the virus occurrence results and to observe its related symptoms. This assay was successfully done using *N. tabacum* plants that inoculated with PVY and showed mild to severe mosaic (Figure 4.4.1). Same PVY related symptoms were reported in a previous study (43). This could prove the reliability of biological assay as a method for viral detection and identification.

In order to confirm the positive results, some positively screened samples were tested at molecular level. RT-PCR analysis of PVY was successful for all samples (5 samples) except two samples which were indicated as highly positive samples using DAS-ELISA (Figure 4.5.2). This failure was not expected as RT-PCR technique considered to be more sensitive than ELISA. But even when we use the most sensitive techniques there is always a chance of getting negative results due to genomic variation of the virus (60). It might be due to the type of primers used (Sprimer & M4) which could prove the finding that this pair of degenerate primers "fall short" in detecting some of potyviruses due to the decay of the consistency of conserved region in potyviruses (11) , or as a result of the existence of inhibitor components in the plant extract (60).

There was a significant difference in the infection percent among the different locations (Table 4.3.1). The highest infection % was in the

locations that belong to An-Nassaiya, Nablus. While in other locations such as Al-Kuffeir, Kufir Qood, Bir al-Basha and Al-Jabriyat no infection was detected in the screened locations. In fact many factors play a crucial role in this variation or could be the causative of this difference in PVY incidence among different locations. Of these, cultivation history, cultivation practices, preventive measurements and temperature are the main ones.

The highest incidence of PVY was detected in the surveyed fields of Nablus district (48%) at An-Nassariya. According to the owners of these fields the source of potato seeds was from the stored tubers of the previous growing season. Thus the inoculum source of the virus could be the seed tubers. Moreover, these samples were the only samples that collected during the fall growing season of 2014, and the temperature in this season is very suitable for the virus activity and multiplication. This might explain the high incidence in that region.

The study has been included fields of one of the agricultural companies in the West Bank (Top Field Company). Potato is one of the main crops that is cultivated in the fields of this company. They usually plant many potato varieties such as Spunta, Arizona, Hermis, Zafira and Desiree. Of these, the Hermis and Desiree are characterized by its high content of dry matter, thus they exported to Jordan in order to be used in potato chips industry. The surveyed fields of Al-kufier, Siris and Al-far'a were affiliated to the “Top Field Company”. All DAS-ELISA screened samples of Alkufier surveyed

fields were PVY negative and showed no symptoms. While the incidence of PVY in Siris and Al-far'a was 5.13 % and 35.71 % respectively, although all the potato seeds were from the same sources. Many virus related symptoms were observed in these fields. Indeed, there was a difference in potato output of each region according to the owners of the company; potato products in Al-Kufier were much higher than those at Siris and Al-far'a. This could be a result of PVY infection particularly in Al-Far'a as the virus incidence was relatively high. It has been noticed that there was a difference in the cultivation practices among these locations. In Al-Kufier the fields were higher equipped and more organized than those of Siris and Al-far'a. In addition to the difference in weather between these locations, as the temperature in Al-Kuffeir is higher than in Al-Fara'a and Siris which are important factors for the virus multiplication as mentioned before.

The same issue was observed in Jenin district. There was a difference in the prevalence of the virus among the different locations of Ash-Shuhada, Bir al-Basha and Al-jabriyat. Even though that they almost has the same weather and shared the same source of tubers.

This may indicate that the prevalence of potato viruses is not consistent from one location to another. Thus, it would be useful to perform further studies on potato viruses' prevalence in the main growing regions of potatoes in Palestine. The obtained information will provide a clear view of areas with the least potato viruses' prevalence which will be suitable for

potato cultivation. Such information would also be useful in developing new strategies for control of viruses occurring in main potato growing regions.

## **5.2 Conclusion and Recommendations.**

The outcome of this study, showed the existence of PVY in the growing fields of potatoes, in varied percentages. The source of the propagated potatoes was influential in the incidence of the virus. Some correlations were drawn regarding to the location of potato fields as well as to the time of planting, suggesting the success in the virus transmissibility by aphids. To our knowledge, this is the first study of symptomology, serological, molecular analysis of one of the viruses that infect potatoes in Palestine. Furthermore this study was able to define the genetic variations among the PVY by using molecular tool (RT-PCR). Even though this study is highly recommended to do more researches on covering the variations among the PVY strains that possibly exist on Palestinian soil. Finally, this study re-emphasized the simplicity, reliability and specificity of using DAS-ELISA for assessment of sanitary status of potato crop regarding to the virus diseases.

## References

1. Salazar, L. F. (2003). **Potato viruses after the XXth century: Effects, dissemination and their control.** In *Crop Protection Department, Seminar Transcript CIP, PO Box* (Vol. 1558).
2. Chiunga, E. (2013). *Viruses occurring in potatoes (Solanum tuberosum) in Mbeya region, Tanzania* (Doctoral dissertation, Helsingfors universitet).
3. Singh, R. P., Valkonen, J. P., Gray, S. M., Boonham, N., Jones, R. A., Kerlan, C., and Schubert, J. (2008). **Discussion paper: The naming of Potato virus Y strains infecting potato.** *Archives of Virology* 153, 1-13.
4. Salazar, L.F. (1996). **Potato viruses and their control.** The International Potato Center (CIP), Lima, Peru. 3-214.
5. Palestinian Central Bureau of Statistics (2013). **Agricultural Statistics, Ramallah– Palestine; available from: <http://www.pcbs.gov.ps/>.**
6. The FAO statistical database-Agriculture. 2014 January 3; <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>
7. Beuch, U. (2013). **Distribution and diversity of Potato mop-top virus in Sweden.** Doctoral Thesis Swedish University of Agricultural Sciences, Uppsala.
8. Mulder, A., & Turkensteen, L. J. (2005). **Potato Diseases: Diseases, Pest and Defects.** *NIVAP, Holland.–2005.–280 p.*

9. Wale, S., Platt, B., & Cattlin, N. D. (2008). **Diseases, pests and disorders of potatoes: a colour handbook**. CRC Press.172p.
10. Nimer, F. ( 1993) *Study Of Potato Virus Y (PVY) and potato virus A (PVA) In Jordan Valley: Inoculum Sources And Incidence*. Uinversity of Jordan. PP1-70.
11. Sokhandan-Bashir, N., Ghasemzadeh, A., Masoudi, N., Khakvar, R., & Farajzadeh, D. (2013). **Degenerate Primers Facilitate the Detection and Identification of Potyvirus-es From the Northwest Region of Iran**. Iran J Biotech, 11(2), 116.
- 12 Sharma, P., Sahu, A. K., Verma, R. K., Mishra, R., & Gaur, R. K. (2013). *Biological and Molecular Characterization of Potato virus Y Infecting Potato (Solanum tuberosum) in India*. Asian Journal of Biological Sciences, 6(5), 257-264.
13. Abbas, M. F., Hameed, S., & Rauf, C. A. (2014). *Presence of New Strain of Potato Virus Y in Pakistan*. International Journal of Agriculture and Biology, 16(5), 941-945.
14. Islam, A. K. M. S., Akanda, A. M., Wazed, M. A., Chewdhery, M. R. A., & Rahman, M. J.(2014). *Performance of fifth generation seed potato against Potato virus Y (PVY)*. scholarly journal of agricultural science,4(2), 78-81.

15. Gray, S., De Boer, S., Lorenzen, J., Karasev, A., Whitworth, J., Nolte, P., ... & Xu, H. (2010). **Potato virus Y: an evolving concern for potato crops in the United States and Canada.** *Plant Disease*, 94(12), 1384-1397.
16. Jones, R., Kumar, S., & Mackie, A. (2003). **Potato virus Y.** *Department of Agriculture, Western Australia. Fact sheet*, (02).
17. Rykbost, K. A., Hane, D. C., Hamm, P. B., Voss, R., & Kirby, D. (1999). *Effects of seedborne potato virus Y on Russet Norkotah performance.* *American journal of potato research*, 76(2), 91-96.
18. Frost, K., Charkowski, A., Gray, S., Crockford, A., & Groves, R. L. (2011). **Management of Potato Virus Y (PVY) in Wisconsin Seed Potato Production.** Division of Cooperative Extension of the University of Wisconsin--Extension.
19. Warren, M., Kruger, K., & Schoeman, A. S. (2005). **Potato virus Y (PVY) and Potato Leafroll virus (PLRV).** *A South African perspective.* *University of Pretoria.* 32pp.
20. Karasev, A. V., Hu, X., Brown, C. J., Kerlan, C., Nikolaeva, O. V., Crosslin, J. M., & Gray, S. M. (2011). **Genetic diversity of the ordinary strain of Potato virus Y (PVY) and origin of recombinant PVY strains.** *Phytopathology*, 101(7), 778-785.

21. Khan, M. S., Hoque, M. I., Sarker, R. H., & Muehlbach, H. P. (2003). **Detection of important plant viruses in in vitro regenerated potato plants by double antibody sandwich method of ELISA.** *Plant Tissue Cult*, 13(1), 21-29.
22. Fakhrabad, F. , Ahmadikhah, A., & Nasrollahnejad, S. (2012). **Identification and detection of Potato virus Y strains by molecular methods in tobacco fields of North Iran.** *International Research Journal of Applied and Basic Sciences*, Vol., 3 (7), 1422-1428.
23. Danci, O., Ziegler, A., Torrance, L., Gasemi, S., & Danci, M. (2009). ***Potyviridae family-short review.*** *Journal of Horticulture, Forestry and Biotechnology*, 13, 410-420.
24. Naidu, R. A., & Hughes, J. D. A. (2003). **Methods for the detection of plant virus diseases.** *Plant Virology in Sub Saharan Africa*, 233-253.
25. Al-Taleb, M. M., Hassawi, D. S., & Abu-Romman, S. M. (2011). **Production of virus free potato plants using meristem culture from cultivars grown under Jordanian environment.** *J Agric Environ Sci*, 11, 467-472.
26. Sawalha, S. (2011). ***Incidence of Viral Diseases on Pepper in Palestine: A Case Study on Potato Virus Y in the Northern Regions of the West Bank Using RT-PCR.*** *Jordan Journal of Agricultural Sciences*, 7(3).



27. Tantowijoyo, W., & Fliert, E. (2006). **All about potatoes: A handbook to the ecology and integrated potato crop management.**
28. Valli, Adrián, García, Juan A, and López-Moya, Juan J (May 2015) **Potyviridae. In: eLS. John Wiley & Sons Ltd, Chichester.** <http://www.els.net> [doi: 10.1002/9780470015902.a0000755.pub3] Valli, A., García, J. A. and López-Moya, J. J. 2015. Potyviridae. eLS. 1–10.
29. SCHOLTHOF, K. B. G., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., ... & Hemenway, C. (2011). **Top 10 plant viruses in molecular plant pathology.** *Molecular Plant Pathology*, 12(9), 938-954.
30. ADAMS, M. J., ANTONIW, J. F., & BEAUDOIN, F. (2005). **Overview and analysis of the polyprotein cleavage sites in the family Potyviridae.** *Molecular plant pathology*, 6(4), 471-487.
31. Miha, M., & Atirib, G. I. (2003). **Overview of Irish potato viruses and virus diseases.** In *Plant Virology in Sub-Saharan Africa: Proceedings of a Conference Organized by IITA: 4-8 June 2001, International Institute of Tropical Agriculture, Ibadan, Nigeria* (p. 334). IITA.
32. Sochor, J., Babula, P., Adam, V., Krska, B., & Kizek, R. (2012). **Sharka: the past, the present and the future.** *Viruses*, 4(11), 2853-2901.
33. Sanfaçon, H. (2005). **Replication of positive-strand RNA viruses in plants: contact points between plant and virus components.** *Botany*, 83(12), 1529-1549.

34. Davie, K., Lacomme, C., & Dickinson, M. (2012). **The biodiversity and epidemiology of Potato virus Y (PVY) in Scotland.** In *The Dundee Conference. Crop Protection in Northern Britain, 2012, Dundee, UK, 28-29. February 2012* (pp. 255-260). The Association for Crop Protection in Northern Britain.
35. Kirchner, S. (2014). **Epidemiology and enhanced control of Potato virus Y in high grade seed potato production.**
36. Chung, B. Y. W., Miller, W. A., Atkins, J. F., & Firth, A. E. (2008). **An overlapping essential gene in the Potyviridae.** *Proceedings of the National Academy of Sciences*, 105(15), 5897-5902.
37. Sanjuan, R., Nebot, M. R., Chirico, N., Mansky, L. M., & Belshaw, R. (2010). **Viral mutation rates.** *Journal of virology*, 84(19), 9733-9748.
38. Cuevas, J. M., Delaunay, A., Visser, J. C., Bellstedt, D. U., Jacquot, E., & Elena, S. F. (2012). **Phylogeography and molecular evolution of Potato virus Y.** *PLoS One*, 7(5), e37853.
39. Roossinck, M. J. (1997). **Mechanisms of plant virus evolution.** *Annual review of phytopathology*, 35(1), 191-209.
40. Abbas, M. F., Hameed, S., Rauf, A., Nosheen, Q., Ghani, A., Qadir, A., & Zakia, S. (2012). **Incidence of six viruses in potato growing areas of Pakistan.** *Pak. J. Phytopath*, 24(1), 44-47.

41. Chen, J., & Adams, M. J. (2001). **A universal PCR primer to detect members of the Potyviridae and its use to examine the taxonomic status of several members of the family.** *Archives of virology*, *146*(4), 757-766.
42. Sokhandan-Bashir, N., Ghasemzadeh, A., Masoudi, N., Khakvar, R., & Farajzadeh, D. (2013). ***Degenerate primers facilitate the detection and identification of potyviruses from the northwest region of Iran.*** *Iranian Journal of Biotechnology*, *11*(2), 115-122.
43. El-Araby, W. S., Ibrahim, I. A., Hemeida, A. A., Mahmoud, A., Soliman, A. M., El-Attar, A. K., & Mazyad, H. M. (2009). ***Biological, serological and molecular diagnosis of three major potato viruses in Egypt.*** *International Journal of Virology*, *5*(2), 77-88.
- 44 Robert, Y., Woodford, J. T., & Ducray-Bourdin, D. G. (2000). **Some epidemiological approaches to the control of aphid-borne virus diseases in seed potato crops in northern Europe.** *Virus Research*, *71*(1), 33-47.
- 45 Batool, A., Khan, M. A., Farooq, J., Mughal, S. M., & Iftikhar, Y. (2011). **ELISA-based screening of potato germplasm against potato leaf roll virus.** *J. Agric. Res*, *49*(1), 57-63.
- 46 Ahmadvand, R., Takacs, A., Taller, J., Wolf, I., & Polgar, Z. (2012). **Potato viruses and resistance genes in potato.** *Acta Agronomica Hungarica*, *60*(3), 283-298.

47. Hull, R. (2009). *Comparative plant virology*. Academic press.
48. Nagy PD and Simon AE (1997) **New insights into the mechanisms of RNA recombination**. *Virology* 235, 1–9.
49. Quenouille, J., Vassilakos, N., & Moury, B. (2013). **Potato virus Y: a major crop pathogen that has provided major insights into the evolution of viral pathogenicity**. *Molecular plant pathology*, 14(5), 439-452.
50. Chikh-Ali, M., Karasev, A. V., Tennant, P., & Fermin, G. (2015). **Potato mosaic and tuber necrosis**. *Virus Diseases of Tropical and Subtropical Crops*, 94-107.
51. Kerlan, C., Nikolaeva, O. V., Hu, X., Meacham, T., Gray, S. M., & Karasev, A. V. (2011). **Identification of the molecular make-up of the Potato virus Y strain PVYZ: Genetic typing of PVYZ-NTN**. *Phytopathology*, 101(9), 1052-1060.
52. Blanco-Urgoiti, B., Dopazo, J., & Ponz, F. (1998). **Potato virus Y group C isolates are a homogeneous pathotype but two different genetic strains**. *Journal of General Virology*, 79(8), 2037-2042.
53. Glais, L., Tribodet, M., & Kerlan, C. (2005). **Specific detection of the PVY N-W variant of Potato virus Y**. *Journal of Virological Methods*, 125(2), 131-136.

54. Piche, L. M., Singh, R. P., Nie, X., & Gudmestad, N. C. (2004). **Diversity among Potato virus Y isolates obtained from potatoes grown in the United States.** *Phytopathology*, 94(12), 1368-1375.
55. Chrzanowska, M. (2001). **Importance of different strains of PVY in potato production and breeding program in Poland.** In *Proceedings of 11th EAPR Virology Section Meeting, Trest (Czech Republic), Potato Research Institute Havlickuv Brod* (pp. 12-14).
- 56 Thresh, J. M. (2006). **Crop viruses and virus diseases: a global perspective.** In *Virus Diseases and Crop Biosecurity* (pp. 9-32). Springer Netherlands.
- 57 van der Vlugt, R. A. (2006). **Plant viruses in European agriculture: current problems and future aspects.** In *Virus diseases and crop biosecurity* (pp. 33-44). Springer Netherlands.
- 58 Hampton, R., Ball, E., & Boer, S. D. (1990). *Serological methods for detection and identification of viral and bacterial plant pathogens. A laboratory manual.* APS press.
- 59 Lima, J. A. A., Nascimento, A. K. Q., Radaelli, P., & Purcifull, D. E. (2012). **Serology applied to plant virology.** *Serological diagnosis of certain human, animal and plant diseases. Rijeka Croácia. InTech*, 71-94.
60. López, M. M., Bertolini, E., Olmos, A., Caruso, P., Gorris, M. T., Llop, P., ... & Cambra, M. (2003). *Innovative tools for detection of plant*

*pathogenic viruses and bacteria*. *International Microbiology*, 6(4), 233-243.

61. Clark, M. F., & Adams, A. N. (1977). *Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses*. *Journal of general virology*, 34(3), 475-483.

62. Chang, P. G. S. (2009). **Plant Virus Diagnostics: Comparison of classical and membrane-based techniques for immunoassay and coat protein sequence characterization for Cucumber mosaic virus and three potyviruses.**

63. Lin, N. S., Hsu, Y. H., & Hsu, H. T. (1990). **Immunological detection of plant viruses and a mycoplasma-like organism by direct tissue blotting on nitrocellulose membranes.** *Phytopathology*, 80 (9), 824-828.

64. Hsu, H., & Lawson, R. H. (1991). **Direct tissue blotting for detection of tomato spotted wilt virus in Impatiens.** *Plant Disease*, 75(3), 292-295.

65. Makkouk, K. M., & Comeau, A. (1994). *Evaluation of various methods for the detection of barley yellow dwarf virus by the tissue-blot immunoassay and its use for virus detection in cereals inoculated at different growth stages.* *European Journal of Plant Pathology*, 100(1), 71-80.

66. Van der Wolf, J. M., Van Beckhoven, J. R. C. M., Bonants, P. J. M., & Schoen, C. D. (2001). **New technologies for sensitive and specific**

**routine detection of plant pathogenic bacteria.** In *Plant pathogenic bacteria* (pp. 75-77). Springer Netherlands.

67. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., ... & Erlich, H. A. (1988). **Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase.** *Science*, 239 (4839), 487-491.

68. Gould, A. R., & Symons, R. H. (1983). **A molecular biological approach to relationships among viruses.** *Annual Review of Phytopathology*, 21(1), 179-200.

69. Alfaro-Fernández, A., Cebrián, M. C., Córdoba-Sellés, C., Herrera-Vásquez, J. A., & Jordá, C. (2008). **First report of the US1 strain of Pepino mosaic virus in tomato in the Canary Islands, Spain.** *Plant Disease*, 92(11), 1590-1590.

70. Kiss, L., Sebestyén, E., László, E., Salamon, P., Balázs, E., and Salánki, K. 2008. **Nucleotide sequence analysis of *Peanut stunt virus* Rp strain suggests the role of homologous recombination in cucumo virus evolution.** *Arch. Virol.* 153:1373-1377.

71. Roossinck, M. J. (2001). **Cucumber mosaic virus, a model for RNA virus evolution.** *Molecular Plant Pathology*, 2(2), 59-63.

72. Sastry, K. S. (2013). **Diagnosis and Detection of Plant Virus and Viroid Diseases.** In *Plant Virus and Viroid Diseases in the Tropics* (pp. 233-353). Springer Netherlands.



جامعة النجاح الوطنية

كلية الدراسات العليا

## الكشف السيرولوجي (المصلي) والجزئي لفيروس البطاطا واي (PVY) في الضفة الغربية

إعداد

نبال يحيى لطفي خديش

إشراف

د. رائد الكوني

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في برنامج العلوم  
الحياتية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، في نابلس - فلسطين.

2016

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### الملخص

فيروس البطاطا "واي" (PVY) يعتبر من أكثر الفيروسات النباتية التي تصيب نبات البطاطا (*Solanum tuberosum*) حول العالم، حيث يحتل المرتبة الخامسة من بين العشرة الأوائل من الفيروسات النباتية الأكثر ضرراً اقتصادياً في جميع أنحاء العالم. في نطاق جمع البيانات حول الفيروسات النباتية في فلسطين، قد أجريت هذه الدراسة للكشف عن الإصابة بفيروس البطاطا "واي" في أهم مناطق زراعة محصول البطاطا في الضفة الغربية - فلسطين، وذلك باستخدام الأساليب السيرولوجية بشكل أساسي الى جانب استخدام الأساليب البيولوجية (الكشف عن الأعراض والتلقيح الميكانيكي) بالإضافة إلى التحليل الجزيئي . تم جمع (255) عينة من أوراق البطاطا المزروعة في (40) حقل متوزعة على مناطق مختلفة في الضفة الغربية خلال مواسم الزراعة لعامي 2014/2015. وقد تم فحص جميع العينات باستخدام الفحص السيرولوجي، وتم أخذ (5) من هذه العينات على الأقل وفحصها على المستوى الجزيئي (RT-PCR) وكشفت النتائج التي تم الحصول عليها من المسوحات الميدانية لحقول البطاطا مجموعة متنوعة من الأعراض الفيروسية مثل الفسيفساء، التقزم، التعضن، الذبول والاصفرار العام، والتي لوحظت في بعض الحقول مشيرة إلى وجود الأمراض الفيروسية. حسب الفحص السيرولوجي تبين أن النباتات التي تم جمعها مصابة بفيروس البطاطا "واي" بمعدل (15.29%)، وقد تم تأكيد وجود الفيروس باستخدام كلا من الفحص البيولوجي والجزئي لبعض النباتات التي تم كشف إصابتها بالفيروس تبعاً لنتيجة الفحص السيرولوجي. والمثير للدهشة، أن جميع عينات نبات البطاطا المصابة كانت من صنف سبونتا عدا عينة واحدة من صنف موندريال، إضافة إلى ذلك أن أعلى نسبة إصابة بالفيروس قد سجلت في الحقول التابعة لمنطقة نابلس بنسبة (48%). إن انتشار الفيروس في الحقول الزراعية الرئيسية لزراعة محصول البطاطا كما ذكرت هذه الدراسة ينبغي أن يكون مقلقاً

ودافعاً لوضع إجراءات لمنع انتشاره. كما تعتبر هذه النتائج مهمة في توفير قاعدة بيانات مفيدة لمزيد من الدراسات لإنشاء نهج إدارة سليم للسيطرة على الأمراض الفيروسية النباتية في فلسطين.