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Phytoviruses in Palestine: Status and Future Perspectives

الفيروسات النباتية في فلسطين: واقع وآفاق مستقبلية

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Abstract

Phytosanitary actions and measurements are nowadays demanded in Palestine to control and limit the pathogenic phytoviruses dissemination throughout propagating materials. Some of the plant viruses can cause significant yield losses which surely affect the national food security. This study aimed to highlight phytoviruses state of the art and researches on plant viruses in State of Palestine. The reviewed subject-related articles and data were obtained from available researches in specialized local and international Journals as well as researches sites (i.e. Research gates; Google scholar; and PubMed). Researches on plant viruses that affecting different economical and agricultural importances such as olives, grapevines, stone fruits, citrus, tomato, and cucurbits were discussed; and their incidence reports were reviewed. It was found that phytoviruses researches and actions towards prevention in Palestine are still in their infancy. None of phytoviruses had been reported to Gaza Strip. This study recommended more researches to be done on the sanitary status on plants with putative high economical and cultural impacts with emphasis on virus and virus-like diseases. To the best of our knowledge, this is the first phytoviruses review study in the State of Palestine. The paper can be considered as a guide and a reference to the field of Phytosanitary services and can be used as a road map to researchers in this field of study.

Keywords: Phytoviruses; Plant diseases; Phytosanitary; State of Palestine.

ملخص

يزداد الطلب في وقتنا الحاضرعلى تشديد إجراءات الرقابة والقياس للصحة النباتية في فلسطين من اجل الحد او السيطرة على انتشار الفيروسات النباتية المسببة للأمراض من خلال الاكثار الخضري. ان بعض تلك الفيروسات قد تتسبب بفقدان كبير في الانتاج النباتي والذي يؤثر سلبا على الأمن الغذائي الوطني. لقد هدفت هذه الدراسة إلى تسليط الضوء على موضوع الفيروسات النباتية في فلسطين، من خلال مناقشة الوضع الراهن للبحوث والدراسات المنشورة حول هذا الموضوع وخاصة من النباتات التي تزرع في دولة فلسطين. لقد تم مراجعة البحوث المنشورة في المجلات المحلية والعالمية وكذلك البيانات المنشورة على مواقع البحوث المعروفة باسم: بوابة البحث؛ وباحث جوجل، والمجلة الطبية. وتم في هذه الدراسة مناقشة الأبحاث على الفيروسات النباتية التي تؤثر على المحاصيل الهامة اقتصاديا وزراعيا كالزيتون والعنب واللوزيات، والحمضيات، والبندورة، اضافة الى القرعيات في العقود الأخيرة. واتضح من خلال الدراسة أن الأبحاث الفيروسية والإجراءات الرامية إلى السيطرة عليها في فلسطين لا تزال في مهدها الاول. اضافة الى انه لم يتم العثور على اي من البيانات او البحوث المتعلقة بالفيروسات النباتية من قطاع غزة. هذا وقد أوصت الدراسة الى ضرورة اجراء المزيد من البحوث للكشف عن الوضع الصحي للنباتات ذات الاهمية الكبيرة من الناحية الاقتصادية والاجتماعية. ووفقا لمعرفتنا، فان هذه الدراسة هي الأولى من نوعها والتي تستعرض موضوع البحوث للفيروسات النباتية في دولة فلسطين. كما ان هذه الورقة تعد بمثابة توجيه ومرجعية للمهتمين بمجال الصحة النباتية وكذلك تعتبر كخارطة طريق للباحثين في هذا المجال.

الكلمات المفتاحية: الفيروسات النباتية في فلسطين، واقع وآفاق مستقبلية.

Introduction

Phytoviruses are sub-microscopic entities where their detection and identifications needs high biotechnological tools (Morris and Dodds 1979; Mowat and Dawson, 1987, Martelli 1993, Al Rwahnih, 2009; Wu *et al.*, 2015). Few laboratories worldwide are considered well equipped to handle these tasks. Besides electron microscopic detection of viruses, several techniques have been used for that such as serological (i.e. ELISA) and molecular tools (PCR and Molecular Hybridization) (Brlansky and Derrick, 1979; Grieco *et al.*, 2002). The dsRNA methods and cytopathological techniques were also used for non specific detection of plant viruses (Martelli, 2013). Thanks to the advances in the sequencing technologies; researchers were enabled to reveal the genetic codes for many viruses and mapping their genomes (Lima *et al.*, 2009; Martelli, 2007). The sequence information was used to classify and nominate accordingly many of these viruses (Alkowni, 2008; Martelli *et al.*, 2012). In the other hand, scientist used viruses as models to understand many aspects of plant physiology, where "Gene silencing" fronted these researches (Eamens *et al.*, 2008). Phytoviruses such as *Tobacco mosaic virus* (TMV); *Tomato spotted wilt virus* (TSWV); *Tomato yellow leaf curl virus* (TYLCV); *Cucumber mosaic virus* (CMV); *Potato virus Y* (PVY); *Cauliflower mosaic virus* (CaMV); *African cassava mosaic virus* (ACMV); *Plum pox virus* (PPV); *Brome mosaic virus* (BMV); and *Potato virus X* (PVX); were listed on top 10 most studied ones due to their scientific and /or economic importance's (Scholthof, *et al.*, 2011).

Palestine; and due to its topography (mountains 1000m elevation above sea level to valley 300m below sea level), and Mediterranean climate (long, hot, dry summer, and rainy warm winter) with annual average of precipitation varied from 10 to 70 cm (depending on the geographic location; south; north; valley or mountain); creates much biodiversity (*fauna* and *flora*) than many other parts of the world and considered as one of the world's hotspot areas (PNA-MEnA, 2006). For that, many viruses could be expected to be there and infecting the plants. As other developing countries, the natural increase rate of Palestinian population reached to (2.96%) in 2012 (PCBS, 2012); which create environmental pressure on the limited natural resources from *biota* and *abiota*. For that, lands, water and food security are always on the top of priority to politicians (*if Israeli occupation's impacts were neglected*) (Anonymous, 1999). According to the PCBS, in 2010 the area of cultivated lands was close to 16% of the total area of Palestinian territory. The total area devoted to plant production is distributed among three main different sub-sectors: Horticulture trees (63.8%), Field crops (23.7%) and Vegetables (12.5%). The Agricultural activities contribution to GDP still recorded low percentage (5.5%), raising the alarming call again for food security in the country and studying the reasons behind that (PCBS, 2012; Alkowni and Srouji, 2009). As previously mentioned, limited water resources, reduction in the arable land (*due to Israelis occupation*), and absence of efficient protection measurements to crops (*farmers ignorance*), may be the major elements for which these can be claimed. For that all, developing and improving agriculture in Palestine by increasing its participation to the GDP, could be the only way to reach the food security. No doubt pests and diseases had great influences on the total yield production, and prices’ instability; the fluctuation in agricultural productions had been noticed in many sectors. This may refers to the way Palestinian farmers are traditionally confronting the pathological and diseased problems. Recent studies on the awareness of farmers towards virus and viral like diseases showed that 92% of olive farmers had no experience in dealing with this issue, even though the study had showed that 96.7% of the surveyed samples expressed their believe that these viruses can cause losses to their crops (Alkowni, 2011). Farmers in Palestine were not able to notice the viral infections effects which were detected in their crops (Djelouah and D'Onghia, 2009), because they were mixed with significant losses in crops yields resulted from chronicle water deficiency and pests invaders.

The main scope of this review study was to report the current status of plant viruses’ existence and spreads in plants that are grown in Palestine. Besides, the ecological impacts of phytoviruses on agricultural economy would be discussed. Actions toward controlling their negative impacts on the commercial crops in Palestine was reviewed as well and updated.

Methodology of data collection

The published data in books, Journals and scientific studies regarding to phytoviruses and phytosanitary actions were obtained from local and international publishers. Data were limited to the period from 1995, when the Palestinian National Authority started to rule, after Oslo agreement in 1993. Research data were obtained from the available sources on the web searching engines as Google scholar; Research Gates and PubMed. These keywords [Plant -Viruses/ diseases/ Phytosanitary- Palestine] were used and filtered to publications started since 1995. All information’s were reviewed and detected viruses from plants grown in Palestine were reported.

Current status of knowledge

The information about phytoviruses in Palestine is scarce due to lack of experts and specialists in this field. Most of the data about viral infections and/or epidemiology's, were come from the neighboring countries that reported the diseases of TYLCV, CTV, CMV, GLRaVD, and many others. These reports were indicated that the same situation could be found on Palestine where the same climate is shared. In addition to that viruses can easily transmit by insect vectors among these countries as they have no boarders (Bar-Joseph *et al.*, 1972; Al Musa, 1982; Czosnek *et al.*, 1990; Abou Jawdah and Shebaro, 1993).

Data about the sanitary status of crops are scanty and erratic (Alkowni *et al.*, 1998). Little was known about pests and pathogens in Palestine where they were common to other Mediterranean countries. Obviously in Palestine, fruit trees were genetically heterogeneous and new varieties were grown together with the old ones; challenging for their adaptation to the new environment and causes failure in many farms as noticed in Alkhader-Bethlehem area. This situation was ascribable due to the following factors: (i) insufficient facilities and equipment for the diagnosis of diseases; (ii) limited numbers of specialized technicians and scientists; (iii) poor organization of nursery and farming activities. The only information about the sanitary status of the crops in Palestine was obtained through out studies carried out by Italian Institutions (IAMB and UBA) in collaboration with the Palestinian Ministry of Agriculture between the years of 1996 to 2001. In these studies several horticultural crops had been investigated for the infection to viruses and virus-like diseases (Alkowni *et al.*, 1998; Jarrar *et al.*, 2000; Saponari *et al.*, 2002; Jarrar *et al.*, 2002; Alayasa *et al.*, 2003; Abbadi *et al.*, 2003). Also the status in the Palestinian grapevine and citrus nurseries during the last 15 years were investigated by researchers at Bethlehem University and some were reported and submitted for publication.

Grapevines were sampled and tested for the viruses GVA, GVB, GFLV, GFkV, GLRaV-1, -2, -3 and -7 by ELISA, meanwhile vein mosaic and vein necrosis virus-like diseases were ascertained by indexing (Alkowni *et al.*,1998). These studies for the assessing the virus disease incidence on grapevines revealed high incidence of viral infections was recorded (82%) especially in local varieties (Table 1). This seems indicative of a long time existence of the virus in the region. The rare and limited detection of GFLV-infected vines in the fields suggested the scarce presence of *Xiphinema index*, its nematode vector. Samples coming from the only rootstocks mother plot, established with material coming from Israeli nurseries, ascertained a low viral infection level (20.3%).

Citrus was also investigated and limited numbers of samples were tested by ELISA to reveal the presence of CTV as a main virus with a high rate of infection in *Shammouti*, *Valencia* and *W. Navel* oranges (Jarrar *et al.*, 2000). In addition to CTV, CPsV was also detected while none of the CVV, CVEV and *S. citri* was found. Considering that, the total infection of the tested pathogens was fairly high (31.6%), it could be higher if viroids and virus-like diseases had been looked for (Jarrar *et al.*, 1999).

Olives, the most cultivated crop in Palestine covering more than 2/3 of total planted area and mainly used for oil production. Sorri is the major cultivated variety representing about 85% of olive trees and used mainly for oil production followed by Local Nabali (Baladi) and Improved Nabali. Other less cultivated varieties are *Mulleesi, Manzinilo, Barenge* and *K18*. A limited number of samples was tested by dsRNA analysis biotechnology to reveal only (38%) of olives as virus free ones (Saponari *et al.*, 2002).

Table (1): List of detected viruses from plants in Palestine.

Plant	Virus tested	Prevalence	Detection methods	References
Grapevine		81.8%		
	Grapevine virus A (GVA) *Grapevine virus B* (GVB) *Grapevine fanleaf virus* (GFLV) *Grapevine fleck virus* (GFkV) *Grapevine leafroll associated virus -1* (GLRaV-1) *Grapevine leafroll associated virus -2* (GLRaV-2) *Grapevine leafroll associated virus -3* (GLRaV-3) *Grapevine leafroll associated virus -7* (GLRaV-7) Grapevine vein mosaic disease Grapevine vein necrosis disease	66.1% 3.7% 1.2% 15.7% 45.6% 8.3% 21.7% 0.2% + +	Serological & Biological assays	Alkowni *et al.*,1998
Stone fruits				
	Apple chlorotic leaf spot virus (ACLSV) *Prunus necrotic ringspot virus* (PNRSV) *Prune dwarf virus* (PDV)	25% 26.7% 13.3%	Serological & Biological assays	Jarrar *et al.*, 2001
	American plum line pattern virus (APLPV)	+	Molecular	Alayasa *et al.*, 2003
	Apricot latent virus from apricot (ApLV)	+	Molecular	Abbadi *et al.*, 2003
Citrus				
	Citru tristeza virus (CTV) *Citrus psorosis virus* (CPsV)	13% 10.5%	Serological (ELISA & DTBIA) & Biological	Jarrar *et al.*, 2002

…continue table (1)

Plant	Virus tested	Preval-ence	Detection methods	Refere-nces
Olives				
	Non specific detection of viruses	66.7%	dsRNA analysis	Saponari *et al.*, 2002
Tomato				
	Cucumber mosaic virus (CMV) Tobacco mosaic virus (TMV) Potato virus Y (PVY) *Tomato yellow leaf curl virus* (TYLCV)	28-93% 15-51% + +	Serological, Molecular & Biological	Sawalha, 2009; 2012; Amro *et al.*, 2014
Cucurbit crops:				
	Squash leaf curl virus (SLCV) Watermelon chlorotic stunt virus (WmCSV)	57.8 % 25%–98%	Molecular	Ali-Shtayeh *et al.*, 2014a; 2014b

Stone fruits were sampled as well and collected from different commercial fruit trees to be tested by ELISA for the presence of stone fruit infecting viruses (Table 1). The detected viruses were PDV, ACLSV and PNRSV with total incidence of infection reached 18.5%, but none of the PDV, ApMV and nepoviruses was detected in any of the sampled stone fruits (Jarrar *et al.*, 2001). Peach had shown the highest percentage of infection (35.6%). The highest incidence was recorded for ACLSV on cherry (25%), PNRSV on peach (26.7%), and PDV on almond (13.3%). All trials to detect these viruses (*Plum pox virus* (PPV), *Apple mosaic virus* (ApMV), *Tomato ringspot virus* (ToRSV), *Raspberry ringspot virus* (RpRSV), *Tomato black ring virus* (TBRV), *Strawberry latent ringspot virus* (SLRSV), *Arabis mosaic virus* (ArMV) and *Cherry leaf roll virus* (CLRV)) were failed (Table 1). In addition to that, *Apricot latent virus* (ApLV) was identified from Palestinian apricots by Abbadi *et al.*, (2003). Another virus *American plum line pattern virus* (APLPV) was isolated from a plum tree and later on was identified (Alayasa *et al.*, 2003). Due to the increase interests on phytoviruses in the country;

several researches developed protocols for detection of the most economical important viruses and virus like diseases such as *Citrus tristeza virus* (CTV); *Grapevine fanleaf virus* (GFLV); *Grapevine leafroll virus*-1,and -3 (GLRaV-1,-3); *Grapevine virus A* (GVA); and *Tomato yellow leaf curl virus* (TYLCV) (Dar-Issa, and Iraki, 2005).

These data were monitored the sanitary status of horticultural main crops, while other researches had been carried to verify the presence of viruses on different vegetables. Tomato as one of high commercial values in Palestine subjected to test for the presence of *Tomato yellow leaf curl virus* (TYLCV) (Sawalha, 2009, Amro *et al.*, 2014). These studies showed that the whiteflies were able to transmit the virus TYLCV on tomato planted in Palestinian fields for 11 days after 48 hrs feeding period acquisition on infected tomato plants. In addition, reduction in the transmission efficiency of the virus was achieved as time progressed until the minimum value after 11 days of virus vector acquisition period (Sawalha, 2009). PCR and ELISA techniques had been used for monitoring the virus presence in this study. Always about TYLCV on Palestinian tomatoes, biological and molecular tools were used to evaluate the TYLCV incidence on different tomato cultivars commercially planted in Palestine. The study revealed that there were no planted cultivars “immune” to TYLCV infection. However, fundamental differences in symptoms development and severity had been discovered (Amro *et al.*, 2014). The study concluded that some cultivars such as tomato with commercial name “3060” could be targeted as promising virus-tolerant ones.

Comparative research study on tomato viruses using ELISA assays revealed that *Tomato yellow leaf curl virus* (TYLCV) was the most dominant disease infecting plants in the northern districts of Palestine (Sawalha, 2012). Its incidence ranged from 28-93%, where the maximum records were in Al-Far'a region of Tobas district. *Cucumber mosaic virus* (CMV) was recorded as the second most prevalent virus on tomato in the same regions as its incidence ranged from 15-51%. *Tobacco mosaic virus* (TMV) and *Potato virus Y* (PVY) was considered insignificant as these viruses were detected in very few tomato samples (Sawalha, 2012). On the other hand, the quantitative effect of four tomato-infecting viruses (TYLCV, CMV, TMV, and PVY) on crop productivity had been evaluated (Sawalha, 2014). The study revealed that simultaneous infections of these four viruses on tomato plants caused severe reduction in the fruit numbers (96%) and weight (93%). Meanwhile single infection by TYLCV alone reduced the fruit number and weight by 77% and 46%; for CMV 63% and 25%; and for TMV 52% and 12% respectively.

Other viruses were isolated for the first time from cucumber; squash and watermelon grown in Palestine (Ali-Shtayeh *et al.*, 2010; Ali-Shtayeh *et al.*, 2012; Ali-Shtayeh *et al.*, 2014a; Ali-Shtayeh *et al.*, 2014b). *Squash leaf curl virus* (SLCV) was the first time reported on infected cucurbits in Palestine. The same was for the *Watermelon chlorotic stunt virus* (WmCSV) infecting watermelons. The SLCV incidence was found up to 85 % in Nablus summer-samples collections; meanwhile it was 98 % in Qalqilia autumn-samples. Molecular tools were used for detection and identifications of these viruses such as Polymerase Chain Reaction (PCR) and Rolling Circle Amplification (RCA) (Ali-Shtayeh *et al.*, 2014b).

Moreover, researches nowadays are conducted to reveal the genetic diversity of many viruses infecting horticultural and vegetable crops in Palestine. For examples Fig virus diseases are surveyed and their symptomatic characteristics were recorded. At least seven viruses and two virus-like diseases were detected in some samples of Fig trees (Alkowni *et al.*, 2015). *Grapevine nepoviruses group A, B* and *C* putatively in their ways to reveal by some researchers due to their destructive symptoms and yield reductions. *Potato virus X* and *Potato virus Y* genetic variations are under investigations by (Abu-Qauod and Alkowni, unpublished data).

Sanitation is one of the effective tools for virus elimination from heavily virus-infected cultivar. The high incidence of virus diseases infections in commercial cultivars makes the use of sanitizing procedures mandatory (Martelli, 1993). Meristem-tips culture alone or in combination with either heat-therapy or chemotherapy can be used for elimination of virus from newly grown tissues. This method is based on the capacity of undifferentiated cells and tissues to escape viral infection due to their rapid growth. Actually, micropropagation proved as a very useful technique for the production of plants free from viruses, viroids and other pests if proper measures and precaution are applied (Beyl & Trigiano, 2014). In Palestine some trials were made to eliminate viruses from infected potatoes (Abu-Qauod and Alkowni, unpublished data). Other trials still in their infant for getting virus free citrus plants (NARC, personnel communications). Whatever the results, at least this branch of biotechnology is now gaining the interests from researchers in Palestinian research labs.

Discussion and Future prospective of phytoviruses in Palestine

Viruses are small entities, affecting all livings (Acheson, 2011), lack of genetic information that encodes apparatus necessary for generation of metabolic energy or for protein synthesis (Cann, 2011). Thus viruses do not grow or undergo divisions; they are completely dependent on their host cells for reproducing their energy (Carter and Saunders, 2013). Some viruses have lipid membrane structure surrounding their capsids, usually belongs to animal-infecting viruses. As plants have rigid cell wall, most of the viruses infecting plants have naked capsids (un-enveloped). Regarding to their nucleic acids structures, about 90% of plant infecting viruses are belonged to Baltimore group IV (Single-stranded positive sense RNAs). This group of viruses replicates their genomes in the host cytoplasm, utilizing their positive sense genome to act as mRNA. In this case the first translated proteins often are functional proteins (*Replicases*, *Helicases*,..etc). Once the virus ensures enough functional and structural proteins, nucleic acids productions will be started produced anti sense RNAs that will act as a template for the positive ones generations (Hull, 2002). This phenomenon will form an intermediate replicative form (RF) structure of dsRNA. That dsRNAs were extensively used for detection and identifications of many plant viruses.

As many viruses had been identified from infected plants, relatively few of them were found to be pathogenesis of their hosts. Some viruses were found latent on their host plants (Savino *et al.*, 1983; Gallitelli and

Savino, 1985; Savino *et al.*, 1984). Latency means ability of the virus to survive and replicate inside the host cells without causing any significant symptoms. These hosts may serve as reservoirs for the virus life cycle (Alkowni, *et al.*, 2001). Viral pathogenesis is not fully understood, but their utilization of the cell mechanism for their replication is ascertained (Flint *et al.*, 2009). Different disease symptoms were observed on infected plants, but they can be grouped into three macroscopic syndromes. (i) Growth retardation: small leaves; mosaic; bushy stunt; yellowing; mottling; reddening; dwarfism; unripe fruits; leaf deformation etc. (ii) Overgrowth: galls; stem swelling; leafroll; rugose wood; ..etc. (iii) Cell death: necrosis, wilting, leaves drop; …etc (Agrios, 2005). It is worth to mention that phytoviruses infecting only the plants, with the exception of “circulative propagative” transmitted viruses that able to replicate inside their insect vectors. These viruses were believed to be as animal viruses transformed to be able to plants infecting ones (Hull, 2002).

Since plant cells are surrounded by thick cell walls, most plant viruses are passively entered to plant cells through grafting, wounds, or by any mechanical ways enable the virus to be in contact directly with plant cells cytoplasm. Another efficient way plant viruses were able to be transmitted to plants is through vectors. Wide variety of organisms used plants as sources of nutrition and some of these organisms, especially invertebrates, act as a virus vector (Agrios, 2005). For examples aphids, mealy bugs, mites, scales, beetles, and nematodes feed by piercing cell walls or by biting.

In additional to their ecological impact on plant health (Boyko, 2005), plant viruses are important from economical point of view as they cause significant yield loss of cash crops and /or agricultural plants during their commercial lifetime. Some estimates put total worldwide damage due to plant viruses as high as US$ $6x10^{10}$ per year (Dale, 1987; Roossink 1999; Shobhana *et al.*, 2014). It is worth to mention that the first virus ever detected was the tobacco mosaic virus due to the damage it did on tobacco in late 19^{th} century. Nowadays more than 77% of known phytoviruses had been initially isolated form cultivated plants (Wren *et al.*, 2006).

Viruses are infecting plants causing different kinds of responses varied from severe damages and reduction in yields to latency. However, latent infections could be hazardous if considering that some viruses using them as a reservoir through their life cycles. In fact, viruses over history co-evolved with their host to a point that they created a balance of co-existence; replicating and spreading with minor damage to hosts (Roossinck, 2013). As human population is growing up exponentially in the last century (Botkin and Keller, 2011), the increase demand on food pushed the monoculture and creation of Agroecosystems which actually disturbing the natural ecosystems. That caused the viruses to test new hosts and try different environments (obviously created by human) causing drastic damages to agricultural crops and shifting the co-existence to the edge, until the nature will do its selection for reaching the eco-Balance (Drosten, 2013; Brown *et al.*, 2006). Till that, we will face the threat of these viruses on our newly cultivated plants with viruses that could be the first isolated and infected them. That is why many crops were found susceptible to many viruses and virus-like agents (phytoplasmas, viroids and unidentified graft transmissible agents) unless they were selected in breeding for their resistant traits. In some cases, infection remains more or less symptomless but in others, very severe symptoms develop upon infection which can cause remarkable yield losses, the unmarketable crops, or exclusion of certain plants that may have social and economical importance’s (Roistacher, 1991).

The wide geographical distribution of viruses could be attributed to dispersal operated by vectors and infected propagative material (Savino, 1998). Man itself is the major responsible for the long distance dissemination of infectious diseases, through the uncontrolled propagation and trading of infected stocks. However, uncontrolled trade and the industrial production of propagating materials had induced the diffusion of phytopathological problems at international level (Martelli and Savino, 1997). The impact of virus and virus-like diseases on the fruit trees industry can be measured in terms of effect on their yield, productivity and quality, although socio-economic consequences should not be disregarded.

For that, immediate actions are needed to be achieved in the country aiming at the final scope to produce and disseminate healthy plants to the environment and particularly to the nurseries. This logic was fully understood and actions had been started many decades in several developed countries, known as “Phytosanitary Actions” (Figure 1). This means the implementation of a “Clonal and Sanitary selection” will be the first step toward applying “Certification program” (Alkowni and Srouji, 2009). The establishment of plots with «healthy» propagated material helps preventing the spread of vector-mediated (insects, nematodes, fungi) or man-mediated (grafting, propagation by cutting) virus-infected plants (Alkowni and Srouji, 2009). In this way, the impact of these diseases on production will be reduced as far as that application is limiting their spread. Clonal and sanitary selection with combination of sanitation is considered as one of the rapid and efficient tools for improvement of productive level of planting.

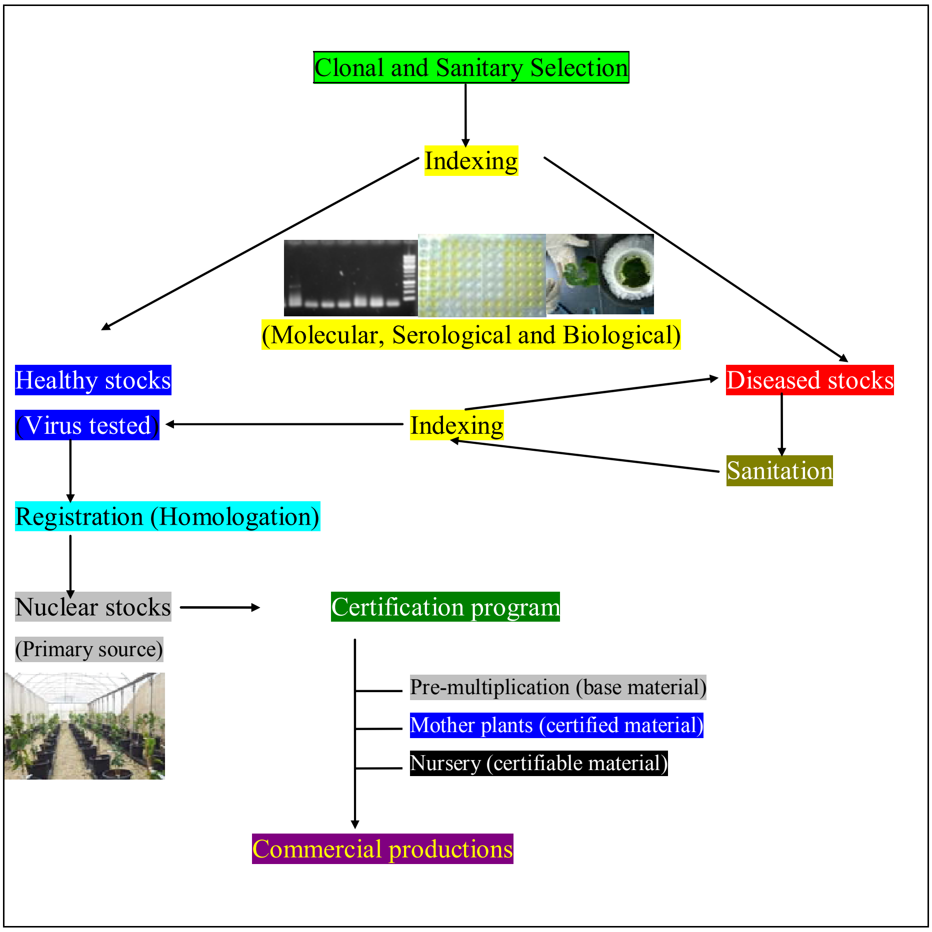

Figure (1): The process of production of healthy propagating material through clonal and sanitary selection followed by the certification program.

The oldest examples of successful certification programs were demonstrated in the United Kingdom, Netherlands, Spain, Italy, and France (Dosba, 1993). In Spain, a program was established in 1977 for the selection, multiplication, and control of fruit tree material (Casallo *et al.*, 1988). In Turkey, modern laboratory facilities were being established and technical staff trained to assay nursery plants for virus and virus-like diseases. Morocco was the first (1977) to produce a certification scheme initially directed to a single stone fruit species (almond), and later extended to all stone fruit species. Meanwhile Tunisia and Algeria were moving towards the adoption of national schemes for stone fruit certification. In Lebanon the technical and legislative protocols for stone fruit certification are now under scrutiny by governmental authorities (Di Terlizzi *et al*., 1998).

In Palestine, to reach this scope, projects were proposed for implementing “phytosanitary actions” since 1999. Within the efforts that National Agricultural research Center / Ministry of Agriculture, in improving the production quantity and quality to meet the standards in the national and international markets, an initiative had been started in 2003 to establish a conservation plot for Citrus varieties. Clonal and sanitary selection for the propagating material free from virus diseases considered as the first step of any expected certification program is also important to improve the productivity of commercial crops (Figure 1).

In other hand, nowadays, FAO is preparing a draft reports on “Sanitary and Phytosanitary (SPS)” by having several meetings with from governmental and non-governmental interest group. It is obvious that this needs well scientific and technical knowledge in virus and virus-like infections and the phytoviruses measurements which based on prevention. In this case knowledge about the current status of sanitary conditions regarding the viruses and virus-like disease on most important crops in Palestine is demanding. Skilled personnel and establishments of technical protocols such the initiative done at UNISCO-Biotechnology Lab in Bethlehem is plausible. What is needed actually, well equipped biotechnology laboratories to be accredited and qualified for doing sanitary work. Universities can collaborate with these tasks through the directed researches on covering out the sanitary status of many commercially important crops will be appreciated. It is worth to say that An-Najah National University / Biology and Biotechnology Department is holding part of these tasks through the Master students program, which aiming to help in revealing the actual status of phytoviruses on upmost important crops such as potato, tomato, grapevine and citrus. This also can be carried on putatively important crops such as Figs and Banana. No doubt for the need of international cooperation in technology's transfer and laboratory accreditation to be recognized internationally is one of the top priorities. Besides, efforts are needed to be paid for putting the suitable legislation regarding to the control of transported propagating materials including phytosanitary certificates; at least for most devastating viruses.

Conclusions and Recommendations

The phytoviruses and related effects on crops protection in Palestine are still in its infancy. Researches on the assessment of the sanitary status of highly economical and social important agricultural crops in Palestine are highly recommended. It is considered as the first step of giving knowledge on the current sanitary status. In parallel; governmental legislation's and rules must be issued and enforced to limit the spread of pathogenic viruses throughout propagating materials. This is collaborative works that need to involve the national scientific institutions (i.e. Universities and research centers) side by side with decision makers in the government. The cooperation with international scientific boards to put realistic and effective measurements for phytosanitary services is highly appreciated. This includes the indeed involvement of the neighboring countries in any international efforts, to harmonize the legislations and avoid contradictions.

Phytosanitary actions should be started in the country by establishments of "germplasm collection plots" or what was called "Clonal and Sanitary selection plots" for plants. In fact this step is the vital and first to be achieved in any implementation of certification programs.

Efforts should be paid on strengthening the biotechnical part in the labs that concerned on detection and identification viruses. In this case, transfer technologies and establishments of accredited labs are highly recommended. Besides, efforts must be paid in developing human resources, by educating and training lab technicians and scientist for detection and identification of plant viruses, as well as doing the process needed for elimination of viruses (especially form native wild cultivars). It is advisable that the scientific institutions' should have to take their responsibilities in establishing programs and specialization that concern on knowledge and skills in Sanitation and phytosanitary Services.

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