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Incidence of potato virus diseases and their significance for a seed certification program in Lebanon

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Summary. Potato fields in the two main production areas of Lebanon, the Bekaa and Akkar plains, were surveyed in two growing seasons for viruses and other pathogens of significance for a potato seed certification program. ELISA tests showed that *Potato virus Y* (PVY) was the predominant virus, followed by *Potato virus A* (PVA), *Potato virus X* (PVX), *Potato virus M* (PVM), *Potato virus S* (PVS) and *Potato leaf roll virus* (PLRV). Of 789 samples tested by ELISA during the two growing seasons, 372 samples were infected by one or more viruses. Single, double and multiple infections represented 75.3, 21.2 and 3.5% of all infected samples, respectively. Incidence of viruses was higher on crops from locally produced uncertified seed-potatoes than on crops from imported certified seed-potatoes. In nucleic acid spot hybridization assays, all 109 samples tested for potato spindle tuber viroid were negative. Other important pathogens of quarantine interest, including *Clavibacter michiganensis*, *Ralstonia solanacearum* and *Synchytrium endobioticum,* were not detected.

Key words: PVY, PLRV, PVA, PVX, PVM, PVS, PSTVd.

Introduction

Potato is the most important field crop grown in Lebanon, with a cultivated area over 14,500 ha and a production exceeding 265,000 tons annually (Jaber, 1997). The two main production areas are the Bekaa plain (68% of total production), at an altitude of 900–1000 m, and the Akkar coastal plain of Northern Lebanon (19%). Though Lebanon has a wide range of microclimates, which makes it possbile to select sites for the production of seed-potatoes, it still relies heavily on imports of seed-potato. Some 12,000 to 18,000 tons of seed-potato is imported each year. At present Lebanon does not have the necessary schemes and facilities for verifying the phytosanitary status of imported planting material.

Virus and viroid diseases are among the diseases of major significance in potato seed production and for certification. They include: *Potato leaf roll virus* (PLRV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus X* (PVX), *Potato virus Y* (PVY), and *Potato spindle tuber viroid* (PSTVd). Other important pathogens include those causing bacterial diseases like *Clavibacter michiganensis* subsp. *sepedonicus* Davis *et al*., *Ralstonia* (*Pseudomonas*) *solanacearum* E.F. Smith, and fungal pathogens like *Synchytrium endobioticum* (Schilbersky) Percival. The maximum permitted level of potato virus diseases var-

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ies from country to country; in the USA it should not exceed 0.5-3% (Slack, 1993; Agrios, 1997), while there is zero tolerance for PSTVd, the two bacteria and the fungus listed above.

Most viruses can be detected by enzyme-linked immunosorbent assay (ELISA) (Petrunak *et al.*, 1991). However, the most sensitive technique for PSTVd is RT-PCR (Shalmoul *et al.*, 1997; Weidemann and Buchta, 1998) followed by nucleic acid hybridization (Salazar *et al.*, 1988; Borkhardt *et al.*, 1994). Singh *et al.* (1988) demonstrated that reverse electrophoresis is sensitive and an acceptable alternative to nucleic acid hybridization.

The symptoms of *S. endobioticum* are obvious and have not been reported in Lebanon for the last few years. Saad and Nienhaus (1969) reported that, in Lebanon, infections by *R. solanacearum* and *C. michiganensis* ssp. *sepedonicus* were rare and localized.

The International Plant Protection Convention (IPPC) stipulated that each country should survey for pests of quarantine significance and submit an updated report on recorded pests. As far as we know, no such survey on virus and viroid diseases on potato has been conducted in Lebanon; the latest published report dates back to 1969 (Saad and Nienhaus, 1969).

This paper presents the results of a survey of potato viruses of major economic importance in Lebanon, reports on other important bacterial and fungal pathogens and discusses their implications for local production of seed-potato.

Materials and methods

Area surveyed and sample collection

A survey was conducted in 1998–1999 in two major cultivation areas: the Bekaa plain and the Akkar coastal plain. A total of 789 samples were collected from 69 fields (Fig. 1). The number of samples from plants showing symptoms suggesting a viral infection were 567 and those from apparently healthy plants were 212. Samples were stored at 4ºC until processed, normally 2 to 5 days.

Serological tests

The standard double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used (Clark and Adams, 1977) for the detection of PVY, PLRV, PVX, PVA, PVS and PVM. IgGs of the first five viruses were kindly provided by the International Potato Center (CIP), Peru, while those of PVM were purchased from Sanofi, France. Leaf samples were extracted (1:10 w/v) in 0.03 M Na_2HPO_4 containing 0.2% diethyldithiocarbamic acid (DIECA). After one hour of substrate incubation, the reaction was detected colorimetrically at A_{405} nm using an ELISA reader (Organon Teknika, Microwell System, the Netherlands). Two wells were used per sample. The test was considered positive when the mean absorbance value of a sample was over twice that of healthy controls.

Nucleic acid hybridization tests

For the detection of PSTVd, the CIP (International Potato Center, Peru) protocol using the nucleic acid spot hybridization (NASH) technique was

Fig. 1. Areas surveyed for the incidence of potato virus diseases.

followed. Leaflets were collected from the upper part of potato plants, 70 samples from Bekaa and 39 from Akkar. One g of tissue was homogenized with 2 ml of 0.2 M K_2HPO_4 containing 10 mM DIECA, 5 mM dithiothreitol and 0.1% Triton x 100. One ml of this mixture was clarified by extraction with an equal volume of phenol/chloroform. After centrifugation at 2000 *g* for 3 min, 5 µl of the upper aqueous extract was spotted on Nylon membranes (N+ Hybond, Amersham Pharmacia Biotech, Little Chalfont, UK). Spotted membranes were incubated at 80°C for 2 hours, wrapped and shipped by rapid mail to the Virology lab at CIP for nucleic acid hybridization using a radioactively labelled probe.

Diagnostic tests for fungi and bacteria

Samples showing symptoms of wilting or chlorosis were collected and tested for fungi and bacteria using common growth media: water agar (WA) and potato dextrose agar (PDA) for fungi, and nutrient agar (NA), King's B (KB) and Miller Schroth medium (MS) for bacteria.

Results and discussion

Potato virus Y was the most common in both seasons. The other viruses varied somewhat in their order of frequency between the two growing seasons. In the first growing season (October 1998–February 1999) out of 246 samples collected, 86 were infected with one or more viruses (Table 1). Double and multiple infections represented 24.4 and 7%, respectively of infected samples (Table 2). Infections by PVY, PVA, PLRV, PVS and PVX were 52.5, 36, 18, 16.2 and 12.8%, respectively. During the second season (April–June 1999), an ELISA test for PVM was included. Of 543 samples collected, 286 were infected with viruses. The relative frequencies of infections by PVY, PVA, PVX, PVM, PVS and PLRV were 82.6, 16.7, 12.2, 5.9, 2.4 and 2.4%, respectively of infected samples; samples with single, double and multiple infections represented 77.6, 19.9 and 2.4%, respectively. In the two growing seasons, PVY-PVA was the most frequently detected mixed infection.

Table 1. Survey of potato viruses in Lebanon. Number of positive DAS-ELISA tests using polyclonal antibodies against six viruses[a] from April 30 to June 23, 1999. Of 543 samples tested, 286 were infected.

Viruses tested	Single infection	Double infection	Triple infection	Quadruple infection
PVS	4			
PVM	4			
PVX	18			
PVY	176			
PLRV	2			
PVA	18			
PVY–PVA		23		
PVY–PLRV		7		
PVX–PVM		1		
PVA–PVX		2		
PVY–PVX		15		
PLRV–PVS		1		
PVY–PVM		8		
PVY–PLRV–PVA			2	
PVY–PVM–PVS			1	
PVY–PVA–PVM			1	
PVY–PVA–PVS			1	
PVY–PLRV–PVM			1	
PVY–PLRV–PVM–PVA				1

[a] PVA = *Potato virus A*; PVM = *Potato virus M*; PVS = *Potato virus S*; PVX = *Potato virus X;* PVY = *Potato virus Y* and PLRV = *Potato leaf roll virus.*

Table 2. Survey of potato viruses in Lebanon. Number of positive DAS-ELISA tests using polyclonal antibodies against five viruses[a] from Oct. 7, 1998 to Feb. 2, 1999. Of 246 samples tested, 86 were infected.

Viruses tested	Single infection	Double infection	Triple infection	Quadruple infection
PVS	6			
PVX	5			
PVY	20			
PLRV	13			
PVA	15			
PVY–PVA		15		
PVY–PVS		2		
PVY–PLRV		2		
PVY–PVX		1		
PVS–PVX		1		
PVY–PVA–PVS			2	
PVY–PVA–PVX			1	
PVY–PVS–PVX			2	
PVY–PVS–PVX–PLRV				1

[a] PVA = *Potato virus A*; PVS = *Potato virus S*; PVX = *Potato virus X*; PVY = *Potato virus Y*; PLRV = *Potato leaf roll virus.*

In general, percent virus infections in crops from locally produced seed-potatoes was higher than that in crops grown from imported seed lots. Based on visual observations, the incidence of virus infections was higher in fields grown from local seed-potatoes than in fields grown from imported ones and was 46.5 vs. 0% in the first growing season and 88.3 vs. 20.7% in the second growing season (Table 3). Depending on the place of production, great variation was observed in the quality of locally produced seed-potatoes. One of the local sources of seed-potato (Kafarkouk, Rachaya, at an altitude of 1200 m) gave very vigorous stands and its visually determined disease incidence was close to nil with only a few plants showing mottling symptoms in two fields (25 ha). Sixteen samples were collected from these plants and tested by ELISA, but all were negative, indicating that, with proper management, good-quality seed-potatoes can be produced in Lebanon. Unfortunately, in most other cases the farmers were not able to trace the source of the locally produced seed-potato, saying that it was either from Bekaa or from Akkar.

In the second growing season, all 125 samples collected in the Akkar plain, from plants with or without symptoms, were infected with PVY alone or mixed with other viruses, mainly PVA, PVM and PVX. In the Bekaa plain, the number of infected samples was 191 out of 358, or 53%, significantly lower than the 100% frequency recorded in Akkar. Several isolated fields or locations in Bekaa, especially the northern Bekaa area, where potato production has recently been introduced, seem to be suitable for production of seed-potatoes. In the first growing season, a low incidence of virus infections was also observed in some fields, in Akkar, that

Table 3. Relation between origin of seed-potato and incidence of virus disease symptoms.

Origin of seed-potato	Number of fields with high disease incidence / total number of fields	
	First season	Second season
Local	7/15 (46.7%)	10/12 (83.3%)
Imported	0/10 (0%)	6/29 (20.7%)

were surrounded by high windbreaks (*Cupressus* sp.). In these fields, potato cultivation replaced old citrus groves.

It is worth mentioning that when imported certified seed-potatoes were grown in the same field along with locally produced uncertified ones, growth of the former appeared more vigorous than that of the latter, which showed uneven growth and somewhat delayed germination. This may, possibly, have been due to a higher incidence of virus infections in these plants and to inappropriate storage conditions between harvesting and planting. In one location, two adjacent potato fields, one with imported and the other with local seed-potatoes, were separated by an asphalt road about 8–10 m wide. About 75–80 days after sowing, the crop grown from imported seed-potatoes showed no symptoms of virus infections, while the crop grown from locally produced seed-potatoes, had a very high incidence of infection (80%). This indicates the importance of having healthy seed stocks, and shows that, under some conditions, aphid-borne viruses spread mainly from sources within a crop. In this particular location, farmers applied two preventive sprays containing chlorpyriphos ethyl (Dursban) to control the potato tuber moth and aphids. However, in other locations, other sources of inoculum, such as neighbouring potato fields, weeds and most probably volunteer potatoes may have an important role in virus dissemination (Jones *et al.*, 1996).

The observed high incidence of PVY infections signals a need for further investigations into the strains of PVY that occur in Lebanon. The PVY^N strain causes less severe symptoms than the other common strain groups (PVY^0 and PVY^C) and plants infected with the former strain could not always be easily recognized (Jones *et al.*, 1996). Therefore, in Lebanon, seed stocks should be carefully tested, in addition to investigating alternative reservoirs of PVY inoculum other than seed-potatoes, especially in the Akkar plain where the incidence of infection by PVY reached 100% in the second growing season. The introduction of potato varieties resistant or tolerant to PVY may play a significant part in reducing yield losses from this virus and thus improving farmers' income.

Nucleic acid spot hybridization assays to detect PSTVd were negative in all the 109 samples tested (39 from the Akkar plain and 70 from the Bekaa).

Concerning bacterial diseases of quarantine significance, no symptoms of *C. michiganensis* or *R. solanacearum* were observed. The only bacterium detected that caused wilting of plants, tuber rot and rotting at the base of the plant was *Erwinia* sp.

As regards fungal pathogens, no symptoms of *S. endobioticum* were observed. A low percentage of plants showed wilting and chlorosis in a field in Turbol. A *Verticillium* sp. was identified as the causal agent. Potatoes infected with this pathogen cannot be used as seed-potatoes. It is worth mentioning that the potato cyst nematode, *Globodera rostochiensis*, was recently introduced into Lebanon; however, a thorough survey has shown that its spread is still very localized (Ibrahim *et al.*, 2000). Therefore, strict internal and external quarantine measures should be taken in order to prevent further spread of the cyst nematode and the entry of any new quarantine pests.

The implementation of a potato-seed production program in Lebanon is possible, but would need to be done under strict phytosanitary control by the concerned authorities. This will not only offer some protection against the introduction of new quarantine pests but will also help increase the income of farmers engaged in seed production.

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