

## Occurrence and distribution of sugar beet viruses in Lebanon

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**Summary.** Field surveys were carried out in the main sugar beet growing areas of Lebanon to assess the occurrence and distribution of viral diseases. A total of 1002 samples from 115 commercial fields were serologically assessed for *Beet Necrotic Yellow Vein Furovirus* (BNYVV), *Beet Yellows Closterovirus* (BYV), *Beet Mosaic Potyvirus* (BtMV), *Beet Western Yellow Luteovirus* (BWYV) and *Cucumber Mosaic Cucumovirus* (CMV). ELISA tests revealed that 39.5% of samples were infected with one or more viruses. BtMV was the most common (56.5%), followed by BYV (29.5%), BNYVV (17%) and BWYV (11%). Mixed infections were detected in 5.2% of the samples. CMV was not encountered. Direct tissue printing of plants infected with BtMV, BYV and BWYV on a nitrocellulose membrane gave very strong positive reactions.

**Key words:** BNYVV, BYV, BtMV, BWYV, tissue blot.

### Introduction

Sugar beet (*Beta vulgaris*) is the most important industrial crop in Lebanon, after tobacco, and represents a major source of sugar for the country. Sugar beet is known to be infected with a number of viruses and virus-like diseases (Duffus, 1973; Heathcote, 1978; Putz *et al.*, 1990). *Beet Necrotic Yellow Vein Furovirus* (BNYVV), *Beet Mosaic Potyvirus* (BtMV) and *Beet Western Yellow Luteovirus* (BWYV), were reported in a preliminary survey of sugar beet virus diseases in Lebanon (Choueiri *et al.*, 1999). The detrimental impact of pathogens on the productivity of sugar beet (Richard-Molard, 1984; Payne and Asher, 1990), the frequent com-

plaints of growers about disease epidemics in sugar beet areas, and the high incidence of Rhizomania in 1998 (Choueiri *et al.*, 1999), necessitated a systematic investigation to determine the health status of sugar beet production in Lebanon. A survey of the incidence, severity and distribution of virus diseases was conducted in 1999 in the Bekaa valley, the major sugar beet growing area in Lebanon. The results of this study are reported and discussed in the present paper.

### Materials and methods

#### Field surveys

Field surveys were undertaken in June, July and August 1999, a period in which the majority of disease symptoms are normally visible under field conditions. The total area under sugar beet in Lebanon for the 1999 season was about 6000 ha. An experiment was designed to collect about 1000 sam-

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ples to represent the sugar beet crop during that season. Samples were collected from three regions of the Bekaa valley. The number of samples from each region was proportionate to the area planted with sugar beet in that region: northern Bekaa, 35 samples from 3 fields, central Bekaa, 425 samples from 54 fields, and western Bekaa, 542 samples from 58 fields. Fields were divided into three groups: A (<5 ha), B (5–50 ha) and C (>50 ha). Five samples were collected from group A, 10 from B and 15 from C. The sampled fields were randomly selected from each region. The samples for laboratory testing consisted of leaves and roots and were collected at random following the “W” pattern as a sampling procedure.

#### Serological tests

**ELISA.** Collected samples were placed in plastic bags, transported to the laboratory in a cold box and kept at 4°C until processing. Double antibody sandwich ELISA (Clark and Adams, 1977) was used for the detection of BNYVV, BYV, BtMV, BWYV and CMV. Sampled leaves were macerated and used for the detection of all viruses except BNYVV, for which rootlets or roots were used. Serological reactants for all the viruses tested in this study were obtained from Loewe Company, Germany. ELISA plates were read with Titertek Multiskan PLUSMK II apparatus.

**Tissue blots.** Tissue blot assays (Hsu and Lawson, 1991) were performed using a polyclonal antibody as purified IgG. Symptomatic leaves of sugar beets suspected of being infected with BtMV, BYV or BWYV were rolled and cut transversely with a sterile razor blade, then gently pressed on nitrocellulose membranes. The membranes were incubated at room temperature for 10 min. They were then immersed in a blocking solution (PBS containing 1% BSA) and incubated at room temperature for 60 min. After three washings of 5 min each in PBS-Tween 0.05%, the membranes were incubated again for 2 h at room temperature with IgG conjugated to the alkaline phosphatase diluted in PBS with 0.5% BSA. The membranes were washed again and stained by soaking in a solution of 14 mg nitroblue tetrazolium (NBT) and 7 mg 5-bromo-4-chloro-3-indolyl phosphate (BCIP) in 40 ml substrate buffer (0.1 M tris, 0.1 M NaCl and 5 mM MgCl<sub>2</sub>, pH 9.5) for 10–15 min at room temperature.

The reaction was stopped by placing the membrane in distilled water for 10 min. The membrane was dried and examined under a binocular microscope.

## Results

### Field symptoms and associated viruses

Symptoms of virus diseases on sugar beet were commonly observed in the fields surveyed. Mosaic symptoms consisting of chlorotic circular spots or rings with green centers on the leaves were observed on plants found to be infected with BtMV alone or in combination with other viruses. Many variations were observed in the type of mottling caused by BtMV, but the mosaic pattern consisted of irregular patches with centers of various shades of green. Plants infected with BWYV showed yellowing of the interveinal areas on older and middle-aged leaves near the leaf tips. Vein clearing and bright yellowing of the veins of younger leaves with necrotic spots were caused by BYV according to the serological tests. A general pale yellowing of the entire leaf blade due to BYV was also very common. Infected leaves became thick, brittle and rough. One plant with clear leaf vein necrosis did not test positive to any of the five viruses tested. BNYVV was characterized by root stunting and abnormal proliferation of fine rootlets on the main tap root with the adhering soil giving it a bearded appearance. This was in line with earlier reports (Tamada, 1975; Hill and Torrance, 1989). The vascular rings of infected roots were darker than those of healthy roots. It should be mentioned that under field conditions only one of all the samples infected with BNYVV showed distinct vein yellowing with necrotic lesions which, as reported by Tamada (1975), is the rare typical and diagnostic symptom of this virus.

### Viruses detected by ELISA

The ELISA tests for viral infections revealed 396 samples (39.5%) with one or more viruses. The incidence of the viruses varied among regions and was lowest (17.1%) in the northern Bekaa, intermediate (35%) in the central Bekaa and highest (44.5%) in the western Bekaa (Table 1). BtMV and BYV were the most widespread, BNYVV and BWYV less common (Fig. 1). The highest virus incidence was that of BtMV (56.5%), followed by BYV (29.5%), BNYVV (17%) and BWYV (11%) (Table 2).

Table 1. Percent incidence of viral infections on sugar beet in three regions of the Bekaa valley in Lebanon.

Region	No. of fields visited	No. of samples collected	No. of infected samples	Incidence (%)
North Bekaa	3.0	35.0	6.0	17.1
Central Bekaa	54.0	425.0	149.0	35.0
West Bekaa	58.0	542.0	241.0	44.5

Table 2. Percent incidence of sugar beet viruses in three regions of the Bekaa valley.

Region	Samples (as %) <sup>a</sup> which reacted positively with antibodies to				
	BNYVV	BYV	BtMV	BWYV	CMV
North Bekaa	0.0	50.0	66.6	0.0	0.0
Central Bekaa	17.4	47.0	42.0	4.0	0.0
West Bekaa	17.0	18.6	65.0	15.7	0.0

<sup>a</sup> Sum of percentages may exceed 100 because some samples were infected with 2 or 3 viruses.



Fig. 1. Distribution of virus diseases on sugar beet in Lebanon

None of the samples tested positive for CMV, indicating that this virus did not occur in the surveyed sugar beet fields in Lebanon. Of the 396 ELISA-positive samples, 343 (86.6%) were infected with one virus. The others (13.4%) had two or more viruses each. BYV+BtMV was the most commonly encountered combination, followed by BtMV+BWYV and BNYVV+BtMV. Three samples were infected with BNYVV+BtMV+BWYV and two with BYV+BtMV+BWYV. The incidence of viruses in the northern Bekaa was lower than that in the other two areas. In the northern Bekaa, 35 samples were collected, of which 6 were infected. Only BYV and BtMV were detected in these samples (Table 2). Many more samples were collected from the central Bekaa due to the larger area cropped with sugar beets. Of the 425 samples collected from the central Bekaa, 149 were virus-infected. Here the incidence of BYV was 47% and that of BtMV 42%, both much higher than BNYVV (17.4%) and BWYV (4%). A heavy infestation by several aphid species during the sampling period (Johnstone and Guy, 1986) may have contributed to the higher incidence. Rhizomania was absent in some localities, but was very high elsewhere probably due to continuous cultivation of sugar beet in this region over many years and the fact that the virus can persist for several years in resting spores of *Polymyxa betae* (Abe, 1987). The western Bekaa had a high incidence of virus infection. Of the 542 samples collected, 241 were infected, mainly with BtMV (65%). The incidence of the other viruses was much lower: BYV 18.6%, BNYVV 17% and BWYV 15.7% (Table 2). Little variation in disease incidence was observed among the localities in this region of the Bekaa.

#### Detection by tissue blot

BtMV, BYV and BWYV were also detected by tissue blotting of young infected leaves on a nitrocellulose membrane. Very strong positive reactions were obtained with blots from virus-infected tissue exposed to IgG conjugated to BtMV, BWYV and BYV, confirming the ELISA results. Infected plant tissues turned violet on the membrane, whereas no change in color was observed in healthy plant tissues. As the tissue blot results were identical with those obtained by ELISA, it is concluded that direct tissue blotting is a reliable

means to detect sugar beet viruses in the leaves (Lin *et al.*, 1990; Hsu and Lawson, 1991; Permar *et al.*, 1992).

#### Discussion

This first systematic survey of sugar beet viruses carried out in Lebanon revealed that the major sugar beet viruses were widespread in the country. Considerable differences were observed in the incidence of virus diseases among the regions: the highest was in the western Bekaa, with an average incidence of 44.5%, followed by central Bekaa, 35%, and northern Bekaa, 17.1%. This lower incidence in the northern Bekaa region is due to the fact that sugar beet cultivation has only recently begun there. Western Bekaa, on the other hand, had the highest incidence of viral diseases as it has been cropped with sugar beets for a long period and with minimal crop rotation, which contributed to the build up of soil-borne diseases, especially rhizomania (Harveson *et al.*, 1996). Rhizomania is the most serious and economically important disease of sugar beet in Lebanon, reducing yields and rendering some large fields useless for sugar beet production. Such heavily infested fields were observed in Maalaka, Terbul, around the sugar beet factory in Anjar, Khiara and elsewhere in the western Bekaa region. The high incidence of rhizomania in these localities was due to: continuous cultivation of sugar beet in the same fields; displacement of infested soil from one field to another by soil adhering to harvesting machines; irrigation with river water contaminated with cystosori of *Polymyxa betae* and cattle feeding on infected beet fields (Hillmann, 1984; Tuitert and Hofmeester, 1994; Harveson *et al.*, 1996). Alteration of the sowing date and the use of sugar beet cultivars tolerant to BNYVV would be the most effective means to control rhizomania (Blunt *et al.*, 1992; Tuitert *et al.*, 1994). Selection and evaluation studies on tolerant varieties are being carried out to determine their performance under field conditions in Lebanon. The beet soilborne mosaic virus (BSBMV), transmitted by *Polymyxa betae*, was not studied during this survey. However, in view of the wide occurrence of the *Polymyxa betae* vector, BSBMV may well occur in Lebanon (Heidel and Rush, 1997).

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Accepted for publication: October 29, 2001