

CUCURBIT YELLOW STUNTING DISORDER VIRUS - A NEW THREAT TO CUCURBITS IN LEBANON

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SUMMARY

Virus diseases are the major cause of economic losses in commercial cucurbit production in Lebanon. Besides mosaic inducing viruses, which have been noted for several years, greenhouse-grown cucumber and melon were often observed with severe yellowing symptoms that started as interveinal mottle on the older leaves and developed into severe yellowing symptoms as the leaves became older. The incidence, in fall crops reached up to 100% in several locations along the Lebanese coast. The presence of *Cucurbit yellow stunting disorder Crinivirus* (CYSDV) was confirmed by the following tests: transmission by *Bemisia tabaci* to cucumber, melon and squash but not to lettuce or to *Physalis floridana*; presence in infected plants of two dsRNAs of about 8 and 9 kbp similar to those reported for criniviruses; observation of a few flexuous particles in leaf dip preparations; cytological alterations observed in infected plants, typical for *Crinivirus* and *Closterovirus* infections; and amplification by RT-PCR, using CYSDV-specific primers, of a DNA fragment that showed 99% nucleotide sequence identity with that of a CYSDV isolate from Spain. This is the first report of CYSDV in Lebanon.

Key words: CYSDV, yellowing, cytology.

INTRODUCTION

Cucurbits are among the major vegetable crops grown in Lebanon. Several virus diseases showing mosaic symptoms were previously reported including *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* type Watermelon (PRSV-W), *Zucchini yellow fleck virus* (ZYFV) and *Cucumber mosaic virus* (CMV) (Makkouk and Lesemann, 1980; Lesemann *et al.*, 1983; Katul, 1986). A recent

survey conducted in the major cucurbit production regions showed that ZYMV was the most frequent and damaging virus followed in frequency by WMV, PRSV-W and CMV (Sobh *et al.*, 1997). This survey also revealed the frequent occurrence of severe yellowing symptoms on older leaves of cucumber, melon and squash in open fields and more often in greenhouses (mainly plastic tunnels).

Yellowing diseases of field- and greenhouse-grown cucurbits are becoming increasingly important in many areas of the world (Wisler *et al.*, 1998). *Beet pseudo-yellowing virus* (BPYV) transmitted by *Trialeurodes vaporariorum* was reported by Duffus (1965), as causing yellowing symptoms in cucumber. The occurrence of yellowing diseases of cucurbits caused by *T. vaporariorum* transmitted viruses that were similar if not identical to BPYV were later reported in different parts of the world (Coffin and Coutts, 1995). These diseases include: *Cucumber yellows virus* in Japan (Yamashita *et al.*, 1979), *Muskmelon yellows virus* in France (Lot *et al.*, 1982) and *Melon yellows virus* in Spain (Jordá-Gutiérrez *et al.*, 1993).

Duffus *et al.* (1986) and Brown and Nelson (1986) reported another type of yellowing disease caused by a virus, *Lettuce infectious yellows virus* (LIYV), transmitted by *Bemisia tabaci* and affecting lettuce, cucurbits, sugarbeet and other hosts in the desert areas of the south-western United States. Hassan and Duffus (1991) described a yellowing and stunting disorder of cucurbits caused by a distinct virus in the United Arab Emirates. This virus was also recently reported from Spain (Célix *et al.*, 1996) and called *Cucurbit yellow stunting disorder virus* (CYSDV). These yellowing viruses are transmitted by whiteflies (either *T. vaporariorum* or *B. tabaci*), have filamentous particles and induce similar cytopathological effects. CYSDV and LIYV are grouped in the genus *Crinivirus*, while BPYV is still considered a member of the genus *Closterovirus* (Wisler *et al.*, 1998, Fauquet and Mayo, 1999).

Lecoq *et al.* (1992) described another yellowing disease of cucurbits that was caused by a *Polerovirus*, *Cucurbit aphid-borne yellows virus* (CABYV), which is transmitted persistently by aphids. In Lebanon, CABYV

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was frequently detected in cucurbit crops grown in the open, while in greenhouses only about 20% of the plants with yellowing symptoms were infected by CABYV, suggesting the involvement of another virus (Abou-Jawdah *et al.*, 1997; Sobh *et al.*, 1997). In this paper, we identify CYSDV as a component of the cucurbit yellowing disease complex in Lebanon, and describe its cytopathological effects in cucumber.

MATERIALS AND METHODS

Transmission and host range. Transmission tests were initially performed with *B. tabaci* collected in a cucumber greenhouse in Jbeil (Byblos) where the crop showed severe yellowing symptoms. These whiteflies were allowed a 48 h inoculation access period on cucumber and squash seedlings at cotyledon stage. Plants were then sprayed with the insecticide imidacloprid (Confidor® 20S4, Bayer, Leverkusen, Germany) and kept in insect proof-cages in a greenhouse.

One cucumber plant that developed yellowing symptoms was used as virus source for further studies. Non-viruliferous *B. tabaci* were reared on broccoli and used for transmission studies. Two transmission methods were used. In method (a) cucumber leaves that developed yellowing symptoms were detached and placed each in a small cage (plastic cup with screens) with 30-50 whiteflies. After an access period of 24 h, the cages containing the whiteflies were used to cover seedlings of melon, cucumber, squash, lettuce and *Physalis floridana*, one cage per seedling, with a minimum of ten seedlings per host. The whiteflies were allowed an inoculation access period of 48 h, then the cages were removed and the plants sprayed with Imidacloprid and maintained in insect-proof cages. In method (b) whiteflies were allowed 72 h acquisition access feeding on squash plants that developed yellowing symptoms in the previous transmission test. About 150 whiteflies were then transferred to insect-proof cages, each containing five seedlings of cucumber, squash, melon, lettuce or *P. floridana*. Following an inoculation access period of 48 h, the plants were sprayed with Imidacloprid and maintained in insect-proof cages. Development of yellowing symptoms on inoculated plants was followed for two months.

Electron microscopy. Crude extracts from symptomatic and healthy plants were observed with a Philips CM10 electron microscope (Eindhoven, The Netherlands) using pyroxilin coated grids and 1% ammonium molybdate, pH 7, as negative stain.

For cytopathological studies, leaf pieces 1 mm across were collected from leaves of infected cucumber plants,

with similar samples from healthy plants as controls. Samples were fixed with glutaraldehyde, postfixed with osmium tetroxide, and embedded in araldite CY212 (Agar Scientific Ltd, Stansted, UK) (Delécolle, 1978). Thin sections were cut with an Ultracut E ultramicrotome (Reichert-Jung, Wien, Austria). Thin sections were stained in 5% uranyl acetate and lead citrate, pH 12.

Extraction and analysis of dsRNAs. dsRNAs were extracted from cucumber, melon and squash plants that developed yellowing symptoms and from their respective healthy controls. Samples of fresh leaf tissue (7 g) were ground in liquid nitrogen and extracted according to Valverde *et al.* (1990). After precipitation with ethanol and sodium acetate the pellet was suspended in 50 µl of distilled water. Aliquots (10 µl) of dsRNA extracts were analysed by non-denaturing electrophoresis in 0.7% agarose gels in tris-acetate EDTA (TAE) (40 mM tris-HCl, pH 7.9, 2.5 mM sodium acetate, 0.5 mM EDTA).

Total RNA extraction, RT-PCR, cloning and sequencing. Total RNA was extracted according to Célix *et al.* (1996). Samples of leaf tissue (0.2 g) were ground in liquid nitrogen. After addition of 400 µl of 100 mM Tris-HCl (pH 8) containing 0.2% sodium dodecyl sulfate (SDS) and 10 mM EDTA, samples were extracted with 1 volume of (1:1) phenol:chloroform. The aqueous phase was adjusted to 2 M LiCl and incubated overnight at 4°C. Samples were then centrifuged at 12,000 g for 15 min and the pellet dissolved in 100 µl of RNase-free water. Aliquots (4 µl) of the total RNA extracts were used in an RT-PCR reaction mixture (20 µl) containing 80 ng of the CYSDV specific primers 410L (5'-TTGGGCATGTGACAT-3') and 410U (5'-AGAGACGGTAAGTAT-3') described by Célix *et al.* (1996). The Access RT-PCR system (Promega, Madison, WI, USA) which performs the reverse transcription and polymerase chain reactions in one buffer system, within a single reaction tube, was used according to the manufacturer's instructions. The RT-PCR reactions consisted of one cycle at 48°C for 45 min, one cycle at 94°C for 2 min, 35-40 cycles at 94°C for 30 s, 42°C for 30 s and 68°C for 70 s and a final extension step at 68°C for 7 min. The amplified products were analysed by electrophoresis in 1.2% agarose gel in TAE buffer.

The amplified fragment obtained by RT-PCR was extracted with two volumes of chloroform and cloned into a pGEM easy vector (Promega). The nucleotide sequence was determined on both strands, with an automated DNA sequencer (Applied Biosystems model 373A, Perkin-Elmer, Foster City, CA, USA). The nucleotide sequence was aligned with highly homologous

sequences in GenBank and EMBL databases using the BLASTN program (Altschul *et al.*, 1990).

RESULTS

Transmission studies. *B. tabaci* was found to transmit the virus from cucumber or squash to cucumber, melon or squash. No symptoms developed on lettuce or *P. floridana*, whatever the virus source. The two methods of transmission used for host range evaluation gave similar results. Yellowing symptoms developed on 60-90% of cucumber, melon and squash seedlings and were prominent four to five weeks after inoculation. Interveneal chlorotic spots appeared on lower leaves and coalesced to give the leaves a bright yellow colour; leaves remained turgid and became brittle. Yellowing became more severe as leaves got older. Symptoms spread upward but never reached the youngest four to five leaves.

Electron microscopy. Observations conducted on crude extracts of infected plants revealed few very flexuous filamentous particles similar to those of viruses in the *Closteroviridae* (Fig. 1). The number of particles observed was too low to estimate their length accurately; similar particles were not observed in healthy plant extracts.

Cytological observations were performed on young and old cucumber leaves showing typical symptoms. In phloem cells (parenchyma or companion cells) numerous vesicles containing fine fibrils were observed (arrows, Fig. 2). Virus particles were also present in the cytoplasm, intermingled with these vesicles (vp, Fig. 2). Some mitochondria appeared to be drastically modified (Fig. 3). Sieve elements were often filled with virus particles, as seen in transverse and longitudinal sections (Fig. 4).

dsRNA analysis. Electrophoresis of dsRNA extracted from infected melon, cucumber and squash samples revealed the presence of two dsRNAs with an apparent size of 8 kbp and 9 kbp (data not shown). These dsRNAs were absent in extracts of the respective healthy controls. However, extracts of healthy controls (especially melon) often had bands of other sizes.

RT-PCR. Electrophoresis of the RT-PCR products from extracts of virus-infected cucumber, melon and squash plants showed a specific DNA of about 460 bp that was absent in extracts of the respective healthy controls (Fig. 5).

The nucleotide sequence of this product was compared with regions of the heat shock proteins (HSP70), homologous genes of three cucurbit yellows inducing

viruses: 99% sequence identity was found with that of the Spanish CYSDV (Accession U67170), but only 59% and 58% sequence identity with LIYV (Accession U15441) and with BPYV (Accession Y15568) respectively.

DISCUSSION

Greenhouse-grown melon and cucumber crops in Lebanon, in recent years, have been severely affected with prominent yellowing symptoms. Farmers and extension officers at first attributed these disorders to either high temperature inside the greenhouse during summer and early fall or to nutritional disorders. However, circumstantial observations suggested a viral nature for the incitant (s) of this disease including low incidence in screened plastic tunnels and very high incidence in adjacent unscreened tunnels. Most farmers reported a yield reduction of 40-60%.

Previous surveys (Sobh *et al.*, 1997) showed that a small proportion of the symptomatic plants collected from greenhouses where the yellowing disease was important reacted positively with a CABYV antiserum, indicating that this aphid-borne virus was, at least occasionally, associated with the yellowing syndrome. However, in the majority of cases, CABYV was not detected in plants with yellowing symptoms, suggesting the presence of another virus (Sobh *et al.*, 1997). Transmission studies conducted in our greenhouses showed that the yellowing symptoms induced by CABYV and those induced by CYSDV infections on cucumber were very similar (data not reported). Symptomatology, transmission by *B. tabaci*, host range limited to cucurbits and virus particle morphology in leaf extracts, indicated that the major yellowing virus incitant in greenhouses in Lebanon was CYSDV.

This identification was confirmed by dsRNA analysis which revealed the presence of two dsRNAs of sizes similar or close to those reported by Célis *et al.* (1996).



Fig. 1. Flexuous virus particle observed in a crude extract of a cucumber leaf showing yellowing symptoms mounted in ammonium molybdate. Bar = 200 nm.

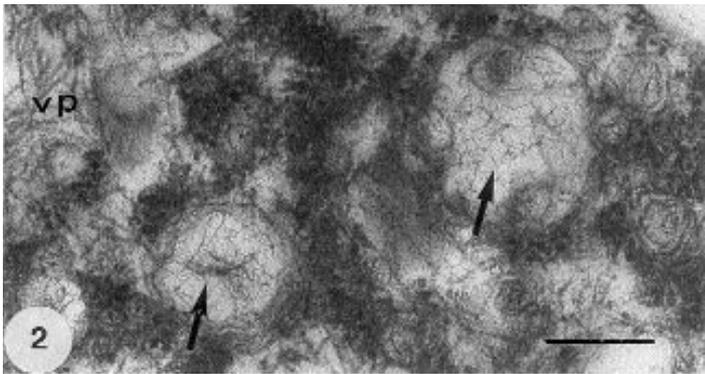


Fig. 2. Cytopathological effects of the yellowing virus in cucumber phloem cells: numerous vesicles containing fibrils (arrows) are observed in the cytoplasm with virus particles (vp) scattered between the vesicles. Bar = 200 nm.



Fig. 3. Cytopathological effects of the yellowing virus in cucumber phloem cells showing vesiculated mitochondria. Bar = 200 nm.

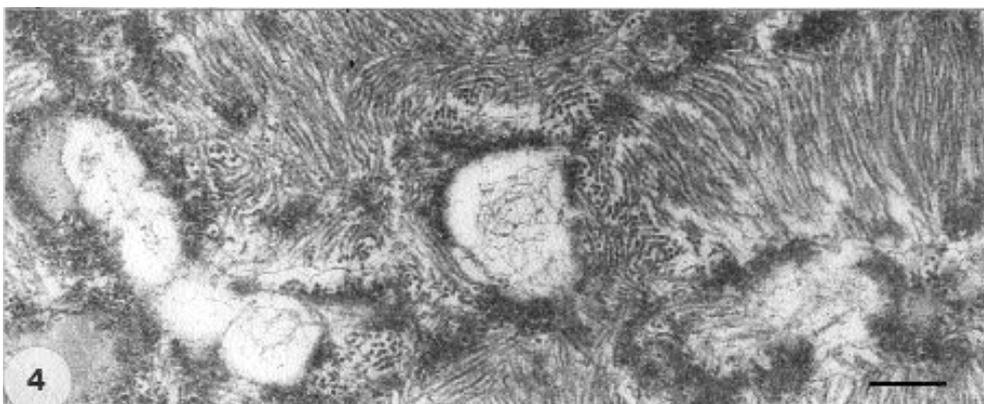


Fig. 4. Numerous virus particles observed in transverse and longitudinal sections in a sieve element of an infected cucumber plant. Bar = 200 nm.

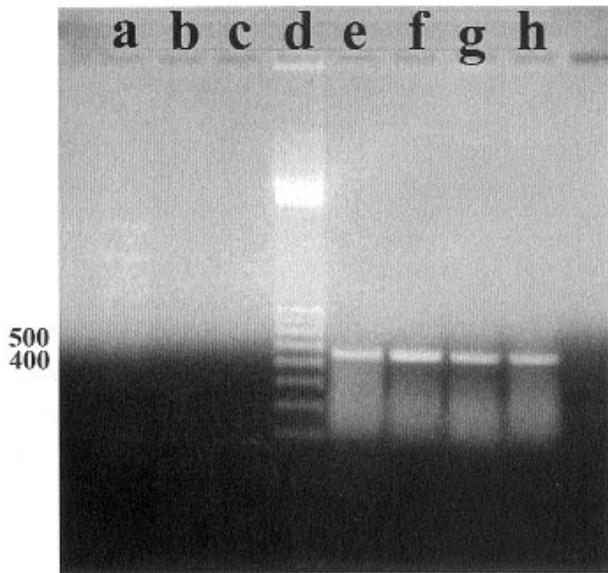


Fig. 5. Agarose gel electrophoresis of RT-PCR amplified products obtained from total RNA extracts of cucurbits using the two CYSDV-specific primers 410U and 410 L described by Céliz *et al.* (1996). Lanes a-c: healthy cucumber, melon and squash, respectively; lane d: molecular marker 100 bp ladder (Pharmacia), with sizes (bp) indicated on the left; lanes e, f: infected cucumber; lane g: infected melon and lane h: infected squash.

Further, RT-PCR using CYSDV-specific primers, and sequencing of the amplified fragment showed a 99% sequence identity with CYSDV from Spain. The dsRNA preparation is quite laborious and requires a large amount of tissue while its results are not always clear (occasional occurrence of bands in the healthy controls). In comparison, RT-PCR is much more versatile and provides more consistent results. Rubio *et al.* (1999) reported that CYSDV isolates could be divided into two divergent groups; the Lebanese isolates apparently belong to group I which includes isolates from Spain, Jordan and Turkey.

The cytological study revealed that CYSDV induces alterations such as vesicles containing fibrils, well known to be associated with infection by members of the *Closteroviridae* (Duffus *et al.*, 1986; Wisler *et al.*, 1998). Vesiculated mitochondria and virion accumulations in sieve elements were also observed.

This is the first report of CYSDV in Lebanon. CYSDV was first observed in the United Arab Emirates (Hassan and Duffus, 1991) and subsequently identified in Spain (Céliz *et al.*, 1996). Recent observations using specific primers indicated that CYSDV has progressively replaced BPYV which was predominant in the Almería region of southern Spain (Jordá-Gutiérrez *et al.*,

1993; Berdiales *et al.*, 1999). This change in virus incidence is to be associated with the increase in *B. tabaci* populations in southern Spain (Berdiales *et al.*, 1999). CYSDV has been recently detected in Turkey, Jordan, Israel, Egypt and Saudi Arabia (Wisler *et al.*, 1998).

The rapid spread of CYSDV in the Mediterranean Basin makes it one of the most typical examples of an emerging virus infecting cucurbits (Lecoq *et al.*, 1998). The impact of CYSDV on cucurbit crop productivity emphasizes the need to implement integrated control strategies to limit the incidence of this disease. In Lebanon, the integration of insect-proof nets, sticky yellow traps and insecticide sprays (mainly Imidacloprid) presently provide a good level of protection.

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REFERENCES

- Abou-Jawdah Y., Sobh H., Fayad A., Lecoq H., 1997. First report of cucurbit aphid-borne yellows luteovirus in Lebanon. *Plant Disease* **81**: 1331.
- Altschul S.F., Gish W., Miller W., Myers E., Lipman W., Lipman D.J., 1990. Basic local alignment tool. *Journal of Molecular Biology* **215**: 403-410.
- Berdiales B., Bernal J.J., Saez E., Woudt B., Beitia F., Rodríguez-Cerezo E., 1999. Occurrence of cucurbit yellow stunting disorder virus (CYSDV) and beet pseudo-yellows virus in cucurbit crops in Spain and transmission of CYSDV by two biotypes of *Bemisia tabaci*. *European Journal of Plant Pathology* **105**: 211-215.
- Brown J.K., Nelson M.R., 1986. Whitefly-borne viruses of melons and lettuce in Arizona. *Phytopathology* **76**: 236-239.
- Céliz A., López-Sesé A., Almarza N., Gómes-Guillamón M.L., Rodríguez-Cerezo E., 1996. Characterization of cucurbit yellow stunting disorder virus, a *Bemisia tabaci*-transmitted closterovirus. *Phytopathology* **86**: 1370-1376.
- Coffin R.S., Coutts R.H.A., 1995. Relationships among *Trialeurodes vaporariorum*-transmitted yellowing viruses from Europe and North America. *Journal of Phytopathology* **143**: 375-380.
- Delécolle B., 1978. Essais de rationalisation des méthodes de préparation d'échantillons végétaux pour la microscopie

- électronique: problème de précipités parasites. *Cellular and Molecular Biology* **22**: 431-436.
- Duffus J.E., 1965. Beet pseudo-yellows virus, transmitted by the greenhouse whitefly (*Trialeurodes vaporariorum*). *Phytopathology* **55**: 450-453.
- Duffus J.E., Larsen R.C., Liu H.Y., 1986. Lettuce infectious yellows virus. A new type of whitefly-transmitted virus. *Phytopathology* **76**: 97-100.
- Fauquet M.C., Mayo, M.A., 1999. Abbreviations for plant virus names. 1999. *Archives of Virology* **144**: 1249-1273.
- Hassan A.A., Duffus J.E., 1991. A review of a yellowing and stunting disorder of cucurbits in the United Arab Emirates. *Emirates Journal of Agricultural Sciences* **2**: 1-16.
- Jordá-Gutiérrez C., Gómez-Guillamón M.L., Juárez M., Alfaro-García A., 1993. Clostero-like particles associated with a yellows disease of melons in South-Eastern Spain. *Plant Pathology* **42**: 722-727.
- Katul L.W., 1986. Occurrence and serological relatedness of five cucurbit potyviruses in Lebanon and Syria. MS Thesis. American University in Beirut, Beirut. Lebanon.
- Lecoq H., Bourdin D., Wipf-Scheibel C., Bon M., Lot H., Lemaire O., Herrbach E., 1992. A new yellowing disease of cucurbits caused by a luteovirus, cucurbit aphid-borne yellows virus. *Plant Pathology* **41**: 749-761.
- Lecoq H., Wisler G., Pitrat M., 1998. Cucurbit viruses: the classics and the emerging. In: McCreight J.D. (ed.). Cucurbitaceae '98, evaluation and enhancement of cucurbit germplasm, pp. 126-142. Alexandria VA, USA. ASHS Press.
- Lesemann D.E., Makkouk K., Koenig R., Natafji-Samman E., 1983. Natural infection of cucumbers by ZYMV in Lebanon. *Phytopathologische Zeitschrift* **108**: 304-311.
- Lot H., Delécolle B., Lecoq H., 1982. A whitefly transmitted virus causing muskmelon yellows in France. *Acta Horticulturae* **127**: 175-182.
- Makkouk K., Lesemann D.E., 1980. A severe mosaic of cucumbers in Lebanon caused by watermelon mosaic virus. *Plant Disease* **64**: 799-801.
- Rubio L., Soong J., Kao J., Falk B.W., 1999. Geographic distribution and molecular variation of isolates of three whitefly-borne closteroviruses of cucurbits: Lettuce infectious yellows virus, Cucurbit yellow stunting disorder virus, and Beet pseudo-yellows virus. *Phytopathology* **89**: 707-711.
- Sobh H., Abou-Jawdah Y., Fayad A., 1997. Survey of cucurbit viruses in Lebanon. In: Khoury W., Bayaa B. (eds.). *Proceedings of the Sixth Arab Congress of Plant Protection, Beirut 1997*, 219.
- Valverde R.A., Nameth S.T., Jordan R.L., 1990. Analysis of double-stranded RNA for plant virus diagnosis. *Plant Disease* **74**: 255-258.
- Wisler G.C., Duffus J.E., Liu H.Y., Li R.H., 1998. Ecology and epidemiology of whitefly-transmitted closteroviruses. *Plant Disease* **82**: 270-280.
- Yamashita S., Doi Y., Yora K., Yoshino M., 1979. Cucumber yellows virus: its transmission by the greenhouse whitefly *Trialeurodes vaporariorum* Westwood, and the yellowing disease of cucumber and muskmelon caused by the virus. *Annals of the Phytopathological Society of Japan* **45**: 484-496.

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