Detection of Varicosavirus and Ophiovirus in Lettuce Associated with Lettuce Big-Vein Symptoms in Brazil

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ABSTRACT

During surveys undertaken from 1998 to 2003 in the major vegetable growing areas of the city of São Paulo green belt, lettuce (*Lactuca sativa*) and endive (*Cichorium endivia*) plants were observed, which showed chlorotic thickening of foliar veins, defective growth and, in some cases, failure to form complete heads. Biological and serological [DAS-Enzyme linked immunosorbent assay (Elisa)] tests together with electron microscope observations, revealed the presence of *Lettuce big-vein virus* and Mirafiori lettuce virus, in these plants both responsible for the lettuce big-vein syndrome.

Additional keywords: *Lettuce big-vein associated virus*, Mirafiori lettuce virus, lettuce, virus transmitted by fungi.

RESUMO

Detecção de Varicosavirus e Ophiovirus associados à síndrome do espessamento clorótico das nervuras da alface no Brasil

Em levantamentos realizados entre 1998 e 2003, nas principais regiões produtoras de alface (*Lactuca sativa*) e escarola (*Cichorium endivia*) no cinturão verde de São Paulo, foram observados sintomas de espessamento de nervuras foliares, clorose, crescimento irregular e ausência de formação da cabeça. Por meio de testes biológicos, DAS-Enzyme linked immunosorbent assay (Elisa) e microscopia eletrônica de transmissão constatou-se a presença do *Lettuce big-vein associated virus* e Mirafiori lettuce virus, responsáveis pela síndrome do espessamento clorótico das nervuras da alface ("lettuce big-vein").

Palavras-chave adicionais: *Lettuce big-vein associated virus*, Mirafiori lettuce virus, alface, virus transmitido por fungos.

In 1998, in the vegetable growing areas of São Paulo's green belt, big-vein symptoms were observed in lettuce (*Lactuca sativa* L.) cultivated in soil or hydroponically, especially during the cooler season. Lettuce big-vein is an important soil-borne viral disease transmitted by the chytrid fungus *Olpidium brassicae* (Wor.) Dang. It is characterized by typical symptoms of thickened veins, foliar clearing areas parallel to the midrib, and vein clearing and shrinking, resulting in poor quality plants with reduced market value. The disease is commonly present at temperatures below 20 °C, a condition frequently observed in temperate climates. The big-vein syndrome is known by this name because its typical symptoms runs parallel to the main vein (Falk, 1997). The cause of this syndrome was attributed to *Lettuce big-vein associated virus* (LBVV) which belongs to the genus *Varicosavirus*. It has rod-shaped particles measuring from 320 to 360 nm and is related to *Tobacco stunt virus* (TStV) (Kuwata *et al*., 1983; Kuwata & Kubo 1984; Vetten *et al*., 1987). According to Roggero *et al.* (2000), a virus from the genus *Ophiovirus*, named Mirafiori lettuce virus (MiLV) was reported to be the causal agent of big-vein symptoms and the formerly described LBVV only caused latent infection, while no synergism was observed with mixed infection by these two unrelated viruses. The MiLV has been also reported on lettuce from European countries, the USA and Japan (Roggero *et al.*, 2000, 2002). Both viruses, MiLV and LBVV, are common in mixed infection in lettuce samples from Northern Italy and France (Roggero *et al*., 2003). Recently, the same situation was reported in Brazil (Colariccio *et al*., 2003; Lima Neto *et al.*, 2004). In surveys undertaken between 1998 and 2003, typical big-vein symptoms (Figure 1A) were observed inthe State of São Paulo that affect different lettuce cultivars, either grown directly in the soil or hydroponically. Leaves and roots of different lettuce cultivars ('Elisa', 'Hortensia', 'Lady', 'Regina', 'Ryder' and 'Verônica'), endive (*Cichorium endivia* L. 'Eliseé' and 'Frevola') and the weeds *Emilia sagitatta* (Vahl) DC., *Galinsoga parviflora* Cav., *Sonchus asper* (L.) Hill and *S. oleraceous* L., were collected in the municipalities of Arujá, Atibaia, Biritiba-Mirim, Cotia, Embu-Guaçu, Guararema, Guarulhos, Itapecerica da Serra, Jacareí, Mogi das Cruzes, Monte Alegre do Sul and Vargem Grande Paulista. All

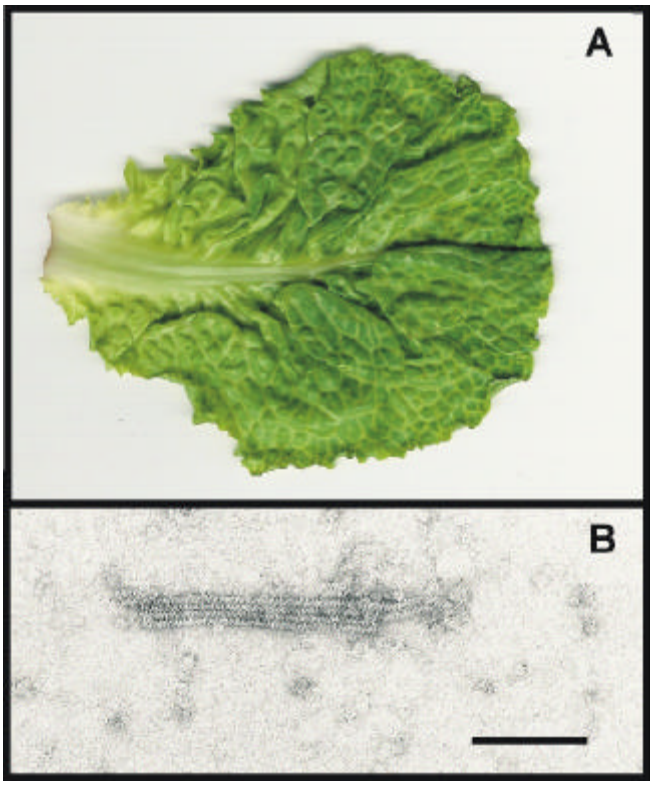

FIG. 1 - Big-vein symptom on lettuce (*Lactuca sativa*) **(A)** and electron micrograph of negatively stained *Lettuce big-vein virus* particles. Bar: 100nm **(B)**

samples were submitted to biological and serological tests, and transmission electron microscope observations. For mechanical transmission, leaf or root tissues from each lettuce cultivar were homogenized with extraction buffer (50 mM phosphate buffer pH 7.0 containing 1 mM Na-EDTA, 5 mM Na-DIECA, 5 mM Na-thioglycolate and 50 mg/ml activated charcoal) (1/5 w/v) in a cooled mortar (Roggero *et al*., 2000). Crude sap was mechanically inoculated on leaves of *Chenopodium amaranticolor* Coste & Ryan, *C. quinoa* Willd., *Nicotiana benthamiana* Domin., *N. occidentalis* Wheeler and of the commercial lettuce cultivars 'Hortensia', 'Regina', 'Verônica'. Soil samples collected in these regions were tested for 'Hortensia' lettuce seedlings germination in greenhouse conditions. All samples (six weeds, nine endives and 171 lettuce cultivars) were tested by DAS-Enzyme linked immunosorbent assay (Elisa), with antiserum specific to MiLV and LBVV, according to Roggero *et al. (*2000).

In the presence of MiLV alone or MiLV and LBVV in mixed infection were detected in the lettuce samples with big-vein symptoms. No symptoms were found in lettuce infected by LBVV alone. Some weeds from lettuce growing areas (Table 1), which showed mosaic symptoms, were infected with LBVV alone. The lettuce symptoms were observed mainly during the cooler season (from June to September), when daytime temperatures range from 22 °C to 18 °C at and nighttime from 16 °C to 10 °C. In the States of São Paulo and Paraná, in Brazil, MiLV alone was detected by Elisa in some lettuce samples with big-vein symptoms, while only LBVV was detected in lettuce samples not showing big-vein symptoms. Leaf fragments of lettuce with symptoms were homogenized at 4 °C in 5% gluteraldehyde in phosphate buffer pH 7.0, negatively stained with 2% uranyl acetate and examined in a Phillips EM 208 electron microscope. Samples of healthy lettuce were used as control.

Results of mechanical transmission were negative for all tested samples, probably due to the fact that experiments had been carried out in the greenhouse at temperatures above 18 °C, as observed by Lot *et al. (*2002). In DAS-Elisa, 51% of the all samples (weeds, endives and lettuce) reacted positively with MiLV or LBVV. From the lettuce samples, 31.2% and 2.15% were infected, respectively, with both viruses or with MiLV alone, whereas 15.6% of the symptomless samples were infected with LBVV (Table 1). These results agree with those described by Lot *et al.* (2002). The only samples that reacted positively against LBVV wee one of *S. oleraceous* with growth reduction and foliar mosaic, and one of endive with mosaic. So far, the occurrence of *S. oleraceous* and *S. asper* as LBVV reservoirs has been reported in several countries (Brunt *et al.,* 1997).

No ophiovirus like-particles were seen in the electron microscope of affected lettuce extracts, although LBVV particles (Figure 1B) were observed in a few symptomatic lettuce samples in which MiLV and LBVV were detected by Elisa. The MiLV particle observation may have failed due to virus lability, as first related by Roggero *et al. (*2000). Thus, the situation encountered in our surveys in the subtropical areas of Brazil (Colariccio *et al*., 2003) is similar to that found in the European temperate climate, with the presence of both viruses. It is worth mentioning that in terms of epidemiological aspects, *O. brassicae* was reported in the central region of Brazil as a vector of the Squash necrosis virus (Lin, 1979; 1984). In a survey carried out in different regions, big-vein symptoms were observed in lettuce, mainly in cultivated higher plains areas and rarely in low flat lands. The fungi vectoring viruses belong to two classes: *Chytridiomycetes* (genus *Olpidium*) and *Plasmodiophoromycetes* (genera *Polymyxa* and *Spongospora*). *Olpidium brassicae* is a vector of minor importance compared to insects, although under favorable conditions it can spread important plant viruses (Costa, 1999) as in the case of LBBV and MiLV (Roggero *et al*., 2000). In São Paulo and Paraná States, MiLV alone was detected by Elisa in some lettuce samples with big-vein symptoms, and LBVV was detected in some symptomless samples (Colariccio *et al*., 2003; Lima Neto *et al*., 2004).

The *O. brassicae* is an obliged parasite whose zoospores commonly infect roots, mainly in wet and cool soil conditions (Costa, 1999). Lettuce big-vein symptoms were observed in conventional and hydroponic lettuce under these conditions during the winter months in São Paulo. The low dissemination of these viruses to neighboring areas is due to the slowmobility of the zoospores and to the

TABLE 1 - Results of DAS-Enzyme linked immunosorbent assay (ELISA) of lettuce (*Lactuca sativa*) samples with virus symptoms collected in São Paulo's green belt regions against antiserum specific to *Lettuce big-vein virus* (LBVV) and Mirafiori lettuce virus (MiLV)

Region	Specie/Cultivar	Sample (H/I)*	Symptom**	DAS-ELISA***	
				LBVV	MiLV
Arujá	*Lactuca .sativa*/Hortensia	4/4	BV	+	+
Atibaia	*L .sativa*/Hortensia	18/5	BV	+	+
	L .sativa/Hyder	5/2	NS	+	-
	L .sativa/Lady	4/2	NS	+	-
	Cichoria endivia/Frevola	1/1	BV	+	+
Biritiba Mirim	*L .sativa* /Ryder	5/0	NS	-	-
	L .sativa /Hortensia	6/1	NS	+	-
	L .sativa /Hortensia	3/2	BV	-	+
	L .sativa /Regina	4/2	BV	+	+
Cotia	*L .sativa* /Hortensia	3/1	BV	+	+
Embu-Guaçu	*L .sativa* /Hortensia	3/3	BV	+	+
Guararema	*L .sativa* /Hortensia	2/2	BV	+	+
Guarulhos	*L .sativa*/Elisa	10/0	NS	-	-
	L .sativa /Elisa	7/3	NS	+	-
	L .sativa /Hortensia	4/1	BV	+	+
	L .sativa /Hortensia	6/5	NS	+	-
	L .sativa /Hortensia	4/4	BV	+	+
	Emilia sagitata	1/0	NS	-	-
	Galinsoga parviflora	1/0	NS	-	-
	Sonchus asper	1/0	NS	-	-
	S. oleraceus	3/1	M	+	-
Itapecerica da Serra	*L .sativa*/ Verônica [h]	7/3	NS	+	-
	C. endivia/Eliseé	3/1	BV	+	+
Jacareí	*C. endivia*/Eliseé	5/1	M	+	-
	L .sativa /Ryder	3/1	NS	+	-
	L .sativa /Ryder	2/1	NS	+	-
	L .sativa /Lady	1/1	NS	+	-
Mogi das Cruzes	*L .sativa* /Elisa	6/6	BV	+	+
M. Alegre do Sul	*L .sativa* /Hortensia	5/0	NS	-	-
	L .sativa/ Hortensia	27/24	BV	+	+
	L .sativa/ Hortensia	10/8	NS	+	-
	L .sativa/ Hortensia	5/2	BV	-	+
V. Grande Paulista	*L .sativa*/ Verônica	6/2	NS	+	-
	L .sativa/ Verônica [h]	11/6	BV	+	+
TOTAL		186/95 (51,0%)			

interdependence of rain, dew and irrigation, as well as its highly unstable condittion outside of the host (Costa, 1999).

The presence in *S. oleraceus* in lettuce crops from São Paulo could be related to spread of big-vein under our conditions as occurred in the North Hemisphere where *S. oleraceous* and *S. aster* were described as LBVV natural reservoirs (Brunt *et al*., 1997). The fact that both weeds are present in Brazil, may indicate they constitute the same reservoirs here.

The incidence of MiLV and LBVV in Brazil (Colariccio *et al*., 2003) and later in Chile (Rosales *et al*., 2004) suggests the possibility of their presence in other temperate and sub-tropical South American countries.

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