

## SHORT COMMUNICATION

VEIN YELLOWING OF *HIBISCUS ROSA-SINENSIS* CAUSED BY *EGGPLANT MOTTLED DWARF VIRUS* IN SOUTHERN ITALYA. De Stradis<sup>1</sup>, G. Parrella<sup>2</sup>, C. Vovlas<sup>3</sup> and A. Ragozzino<sup>4</sup><sup>1</sup>Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126, Bari, Italy<sup>2</sup>Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055, Portici, Italy<sup>3</sup>Dipartimento di Protezione delle Piante e Microbiologia Applicata, Via Amendola 165/A, 70126, Bari, Italy<sup>4</sup>Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Via Università 100, 80055, Portici, Italy

## SUMMARY

*Eggplant mottled dwarf virus* (EMDV) was detected for the first time in southern Italy in several *Hibiscus rosa-sinensis* L. plants with vein yellowing. The virus was identified by the reactions of a herbaceous host range, cytopathology and serology. Virions were observed only in the nuclei of infected cells where they accumulated between the inner and outer lamella of the nuclear envelope. The virus was identified by an antiserum to a potato isolate of EMDV both in immunoelectron microscopy tests and in leaf sap with indirect ELISA. A limited survey revealed that EMDV occurs in naturally infected hibiscus in Campania, Calabria and Sicily (southern Italy). Some aspects of the epidemiology of EMDV are briefly discussed.

**Key words:** EMDV, rhabdovirus, cytopathology, immunodecoration.

*Hibiscus rosa-sinensis* L. (family *Malvaceae*) is an ornamental shrub reproduced by rooted cuttings, widely grown outdoors in southern Italy. In 2005 and 2006, several plants of different *H. rosa-sinensis* cultivars, grown in Campania, Calabria and Sicily were observed which showed yellowing of the veins, diffuse mottling and occasional distortion of the youngest leaves (Fig. 1). Comparable symptoms were also observed on small potted *H. rosa-sinensis* plants imported from abroad, on sale in a supermarket in Campania.

From sap extracted in the presence of 0.1 M phosphate buffer pH 7.2 from young leaves of a number of symptomatic plants a virus was consistently transmitted to a range of herbaceous hosts. *Nicotiana occidentalis* and *N. clevelandii* reacted with yellow local lesions followed by systemic vein clearing, mottling, and stunting, whereas *N. benthamiana* and *N. glutinosa* developed only systemic symptoms. *Gomphrena globosa* reacted lo-

cally with reddish local lesions. No symptoms were observed in *N. rustica*, *Chenopodium quinoa*, *C. amaranticolor* and *Citrullus vulgaris*. Systemic symptoms in *Nicotiana* spp. appeared in many instances about one month after inoculation and resembled very much those induced by *Eggplant dwarf mosaic virus* (EMDV) (Martelli and Rana, 1970; Martelli and Russo, 1973), a nucleorhabdovirus first recorded from Italy in 1969 (Martelli and Cirulli, 1969).

Electron microscope observations of leaf dips and ultrathin sections of symptomatic *Nicotiana* spp. and *H. rosa-sinensis* revealed the consistent presence of rhabdovirus-like particles *ca.* 230 × 70 nm in size in the analyzed samples, thus confirming the indications given by biological assays. In thin-sectioned cells of *H. rosa-sinensis* virus particles were localized in perinuclear position between the inner and outer membrane of the nuclear envelope (Fig. 2). Most of the particles were bullet-shaped, others were bacilliform; both were oriented perpendicularly to the inner perinuclear membrane. Some particle aggregates were also observed in the nucleoplasm in membrane-bound pocktes. Also the ultrastructural features tallied with those characterizing EMDV infections (Martelli and Russo, 1973).

In immunoelectron microscopy tests (Milne, 1984), virus particles were clearly decorated with a polyclonal antiserum to an Iranian potato isolate of EMDV (Fig. 3), kindly provided by Dr. B.E.L. Lockhart. The same serum detected the virus, by indirect ELISA, in several symptomatic leaf samples from Campania (5 samples, including two on sale in a supermarket), Calabria (15 samples) and Sicily (2 samples).

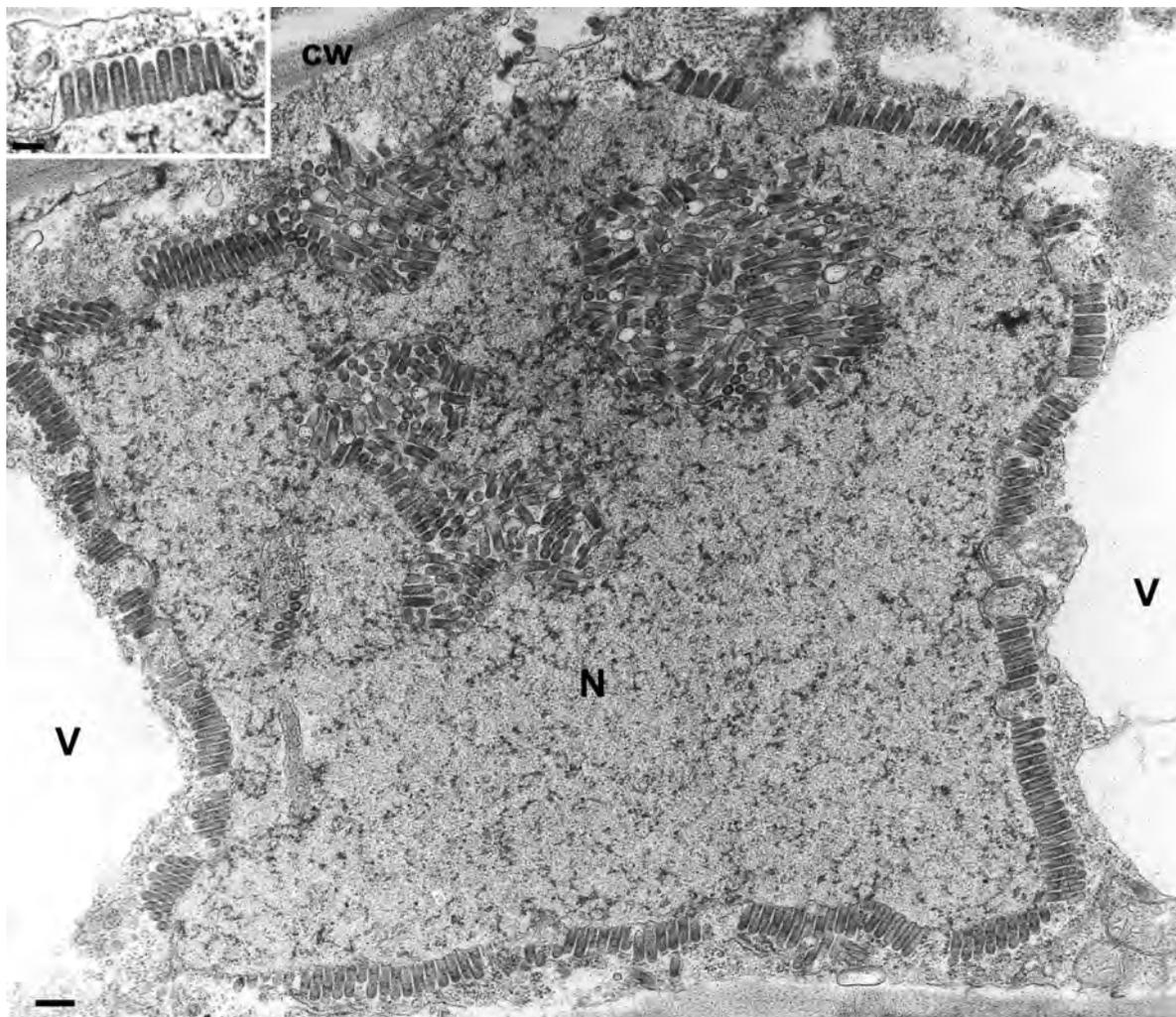
A similar disease of *H. rosa-sinensis* has previously been reported from Morocco, Tenerife and Rhodes and the associated virus was identified as an isolate of EMDV (Plavsic and Milicic, 1984; Lockhart, 1987). Among viruses infecting *H. rosa-sinensis*, *Hibiscus chlorotic ring spot virus* (HCRSV) may elicit vein banding symptoms, resembling somewhat those caused by EMDV. However, HCRSV has small isometric particles, does not infect *Nicotiana* spp. and does not seem to occur in the Mediterranean basin (Raju, 1985).

Natural spread of EMDV has been reported in several Mediterranean countries on annual crops like pepper, tomato, potato, cucumber, tobacco, eggplant and

Corresponding author: A. De Stradis  
Fax: +39.080.5442911  
E-mail: a.destradis@ba.ivv.cnr.it



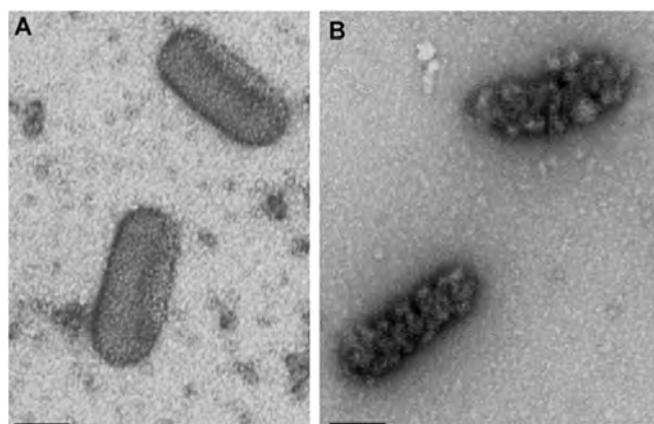
**Fig. 1.** Symptoms shown by EMDV-infected leaves of *Hibiscus rosa-sinensis*. A healthy leaf on the left hand side.



**Fig. 2.** EMDV-infected hibiscus cell showing virus particles budding from the nuclear membrane and in membrane-bound pockets in the center of the nucleus. Inset shows a close-up of a group of budding virions. N, nucleus; V, vacuole; cw, cell wall. Bar=300 nm, inset bar =150 nm.

muskmelon, indicating that a vector is likely involved in the transmission of the virus, though with a low efficiency (Martelli, 1969; Martelli and Hamadi, 1986; Lockhart, 1987; Ciuffo *et al.*, 1999; Katis *et al.*, 2000; Kostova *et al.*, 2001; Aramburu *et al.*, 2006; Mavric *et al.*, 2006).

EMDV is transmitted in nature by leafhoppers. *Anaceratagallia laevis* and *A. ribauti* were identified as vectors of the virus isolate inducing cucumber toad-skin disease in France (Della Giustina *et al.*, 2000) and *Agallia vorobjevi* transmitted EMDV to eggplant in experimental trials carried out in Iran (Babaie and Izadpanah, 2003). Whereas *A. vorobjevi* does not seem to occur in Italy and in other Mediterranean countries where EMDV spreads naturally in some annual crops, the two *Anaceratagallia* species are endemic in Europe where they may be involved in virus transmission also in countries other than France. However, since *Hibiscus* spp. are vegetatively propagated, the multiplication and marketing of infected stocks is strongly suspected as being the main cause of the disease observed in *H. rosa-sinensis* in southern Italy.



**Fig. 3.** Uranyl acetate mounts of virus particles from infected hibiscus leaves (A) and virus particles decorated with the antiserum to EMDV (B). Bars = 100 nm.

This appears to be the first record of natural EMDV infections in *H. rosa-sinensis* in Italy. Although the low percentage of infected plants, regardless of the host considered, and the low efficiency with which EMDV is transmitted by vectors, does not make this virus a pathogen of primary economic importance, yet the adoption of measures aimed at preventing the introduction and distribution of infected stocks appears desirable. This would reduce the establishment of perennial reservoirs of the virus and, in the long run, reduce the inoculum potential.

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#### REFERENCES

- Aramburu J., Galipienso T., Tornos T., Matas M., 2006. First report of Eggplant mottled dwarf virus in mainland Spain. *Plant Pathology* **55**: 565.
- Babaie G.H., Izadpanah K., 2003. Vector transmission of eggplant mottle dwarf virus in Iran. *Journal of Phytopathology* **151**: 679-682.
- Ciuffo M., Roggero P., Masenga V., Stravato V.M., 1999. Natural infection of muskmelon by eggplant mottled dwarf rhabdovirus in Italy. *Plant Disease* **83**: 78.
- Della Giustina W., Javoy M., Bansept P., Morel E., Balasse H., Goussard N., Passard C., 2000. Les cicadelles du genre *Anaceratagallia* vectrice du virus responsable de la maladie de la peau de crapaud du concombre. *PHM-Revue Horticole* **420**: 40-43.
- Katis N.I., Chatzivassiliou E.K., Clay C., Avgelis I., Manousopoulos I., Lecoq H., 2000. Occurrence of eggplant mottled dwarf nucleorhabdovirus (EMDV) in tobacco and cucumber crops in Greece. *Phytopathologia Mediterranea* **39**:319.
- Kostova D., Masenga V., Milne R.G., Lisa V., 2001. First report of Eggplant mottled dwarf virus in cucumber and pepper in Bulgaria. New Disease Report <http://www.bspp.org.uk/ndr/jul2001/2001-30.asp>
- Lockhart B.E.L., 1987. Evidence for identity of plant rhabdoviruses causing vein-yellowing diseases of tomato and *Hibiscus rosa-sinensis*. *Plant Disease* **71**: 731-733.
- Martelli G.P., 1969. Bacilliform particles associated with mottled dwarf of eggplant (*Solanum melongena* L.). *Journal of General Virology* **5**: 319-320.
- Martelli G.P., Cirulli M., 1969. Mottled dwarf of eggplant (*Solanum melongena* L.), a virus disease. *Annales de Phytopathologie* **1**: 393-397.
- Martelli G.P., Rana G.L., 1970. Mechanical transmission of Eggplant mottled dwarf virus (EMDV). *Phytopathologia Mediterranea* **9**: 187-191.
- Martelli G.P., Russo M., 1973. Eggplant mottled dwarf virus. *CMI/AAB Descriptions of Plant Viruses*, No. 115.
- Martelli G.P., Hamadi, 1986. Occurrence of Eggplant mottled dwarf virus in Algeria. *Plant Pathology* **35**: 595-597.
- Mavric I., Tusek Znidaric M., Virscek Marn M., Dolnicar P., Mehle N., Lesemann D.E., Ravnikar M., 2006. First report of Eggplant mottled dwarf virus in potato and tomato in Slovenia. New Disease Report. <http://www.bspp.org.uk/ndr/july2006/2006-09.asp>
- Milne R.G., 1984. Electron microscopy for the identification of plant viruses in in vitro preparations. *Methods in Virology* **7**: 87-120.
- Plavsic B. and Milicic D. 1984. Rhabdovirus-like particles associated with vein yellowing of *Hibiscus rosa-sinensis* L. *Acta Horticulturae* **164**: 41-43.
- Raju B.C., 1985. Occurrence of chlorotic ringspot virus in commercial *Hibiscus rosa-sinensis* cultivars. *Acta Horticulturae* **164**: 273-280.

