VEIN YELLOWING OF HIBISCUS ROSA-SINENSIS CAUSED BY EGGPLANT MOTTLED DWARF VIRUS IN SOUTHERN ITALY

A. De Stradis¹, G. Parrella², C. Vovlas³ and A. Ragozzino⁴

¹Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126, Bari, Italy
²Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055, Portici, Italy
³Dipartimento di Protezione delle Piante e Microbiologia Applicata, Via Amendola 165/A, 70126, Bari, Italy
⁴Dipartimento 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 locally with reddish local lesions. No symptoms were observed in N. rustica, Chenopodium quinoa, C. amaranthicolor 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 pockets. 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...
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.
musk melon, 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; Giuffo 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 leaf hoppers, 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](image)

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.

**ACKNOWLEDGEMENTS**

The gift of the antiserum to EMDV by Dr, B.E.L. Lockhart, Department of Plant Pathology, University of Minnesota, St. Paul, MN, USA is gratefully acknowledged. We also thank Prof. G.P. Martelli for helpful comments and revision of the manuscript.

**REFERENCES**


