

UDC 576.858.8:582.572.228 = 20
Original scientific paper

ETIOLOGY OF RINGSPOT SYMPTOMS IN *DRACAENA SURCULOSA* LINDL.

MLADEN KRAJAČIĆ and NADA PLEŠE

(Department of Botany, Faculty of Science, University of Zagreb)

Received March 5, 1993

Prominent virus-like ringspot symptoms were observed on the leaves of *Dracaena surculosa* Lindl. Attempts to transmit a casual virus by mechanical inoculation on some conventional test plants remained unsuccessful. However, leaf squash preparation revealed filamentous virus particles, as well as structures which could be parts of »pin-wheel« inclusions. dsRNA, corresponding approximately to the replicative form of RNA of potyviruses, was isolated from symptoms bearing leaves. The results obtained suggest that the ringspot symptoms on *D. surculosa* could be provoked by a potyvirus infection.

Introduction

Conspicuous regular chlorotic rings and spots, both of 0.5 to 1 cm in diameter (Fig. 1), were observed on the leaves of *Dracaena surculosa* Lindl. growing in the glasshouse of the University Botanical Garden in Zagreb. The peculiarity of these virus-like symptoms suggested that it would be worth-while to examine the etiology of the symptoms expressed, as well as to identify a possible causal agent. This short communication presents the results of these investigations.

Materials and Methods

Supposing that it is a question of virus infection, we tried to isolate the agent from *D. surculosa* by mechanical inoculation on a series of conventional virus test plants. For transmission experiments the inocula were prepared by grinding young symptom bearing leaves of *D. surculosa* in 0.06 M phosphate buffer pH 7.0, which contained 0.3% sodium ascorbate or 0.2% sodium diethyldithiocarbamate. The transmission experiments were conducted early in spring and autumn.

Searching for virus particles in *D. surculosa*, extracts of leaf pieces from the region of chlorotic alterations and also from the leaf blade without symptoms were stained in 2% sodium phosphotungstate. For submicroscopical observation ultrathin sections through the leaf tissue in the region of chlorotic rings and spots were prepared by the procedure by Pleše and Wrischer (1978). Preparations were examined in a Siemens Elmiskop I.

Isolation of dsRNA was accomplished following the procedure described by Krajačić and Lorković (in press). Ten-gram portions of frozen leaf tissue of *D. surculosa* showing symptoms were ground in 15 ml of STE extraction buffer. After phenol and chloroform extraction of total nucleic acid, dsRNA was further purified by chromatography on a CF-11 cellulose column, concentrated by ethanol precipitation and analyzed by agarose gel electrophoresis and ethidium bromide staining. dsRNAs of cucumber mosaic virus, potato virus Y and turnip yellow mosaic virus were used as molecular weight standards.

Results and Discussion

In transmission experiments there was no response in any species of herbaceous test plants used. The agent could not be mechanically transmitted either with sodium ascorbate or with sodium diethyldithiocarbamate as additives to the inocula. Different vegetational seasons had no influence on the negative result of transmission tests, too.

However, leaf squash preparations of investigated *D. surculosa* with ringspot symptoms revealed a number of elongated, slightly flexible virus particles (Fig. 2). The particles were present in the leaf extracts from the region of virus symptoms, and also in the leaf sap from the normal green leaf area. In addition, only in squash preparations from the region of ringspot symptoms, could structures which looked like the parts of pinwheels (Fig. 3) be observed. The possible submicroscopical alterations in virus infected leaf cells of *D. surculosa* could not be determined, because, in the ultrathin sections prepared the tissue was destroyed so much that it was unsuitable for electron microscopical analysis. This was probably due to the peculiarity of the vacuole content which during the preparation of sections caused a total cell destruction covering all cytoplasmic structures.

A dsRNA analysis revealed a very faint band with the electrophoretic mobility corresponding approximately to those of potyviruses dsRNA. As Valverde et al. (1986) pointed out, different viral groups were clearly distinguishable by the similar size of the replicative RNA forms of their members, which differ for each group. Additional dsRNAs of faster mobility than the replicative forms have been found for several

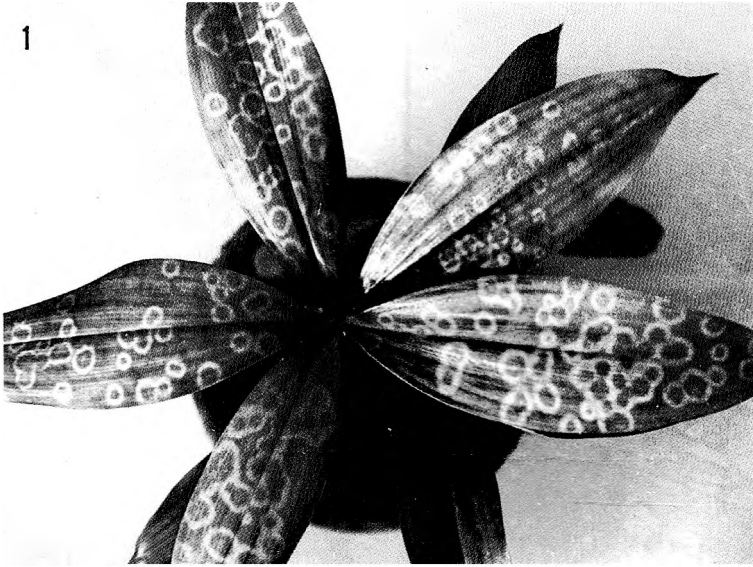


Fig. 1.

▲
Fig. 1. Chlorotic ringspot symptoms on leaves of *Dracaena surculosa* Lindl.

Fig. 2. Electron micrograph of filamentous virus particles in leaf extract of *Dracaena surculosa* with ringspot symptoms. Bar represents 500 nm. ►

Fig. 3. Submicroscopical structures similar to the parts of »pinwheel« inclusions (arrows) in leaf extract of *D. surculosa*. Bar represents 500 nm. ►

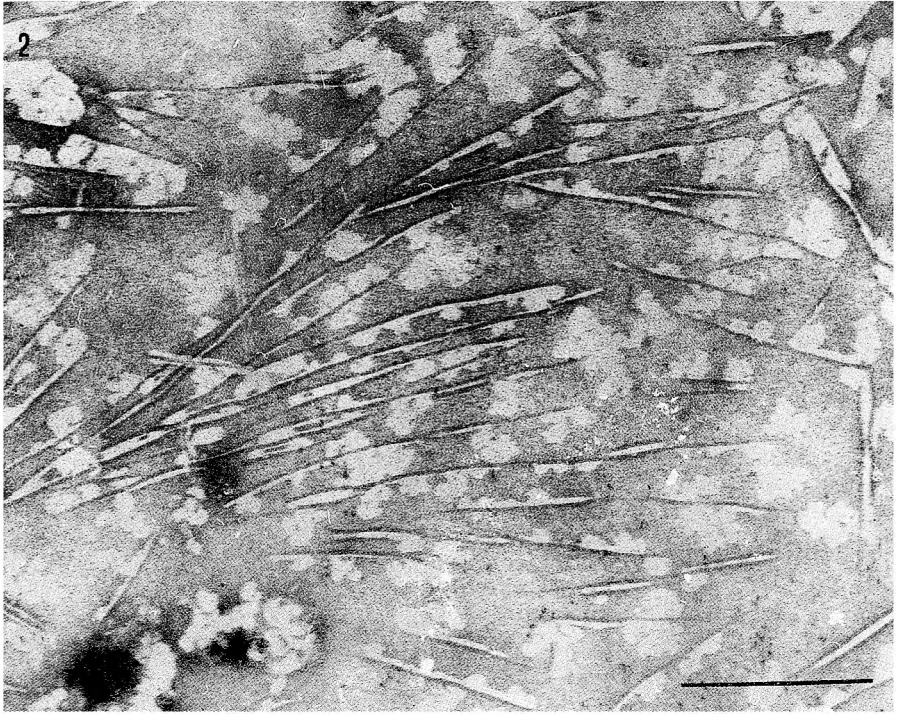


Fig. 2.

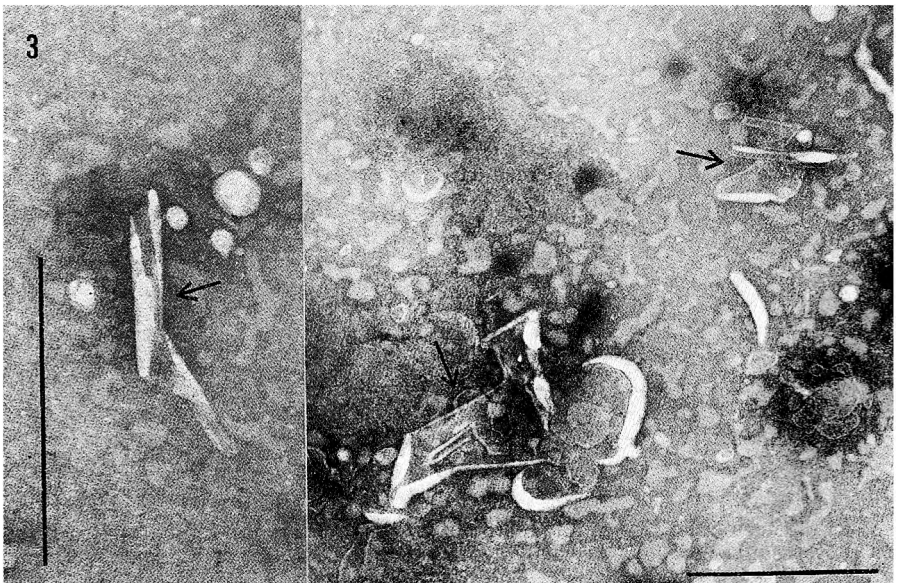


Fig. 3.

plant viruses (Dodds and Bar-Joseph 1983, Gildow et al. 1983, Lorković and Krajačić (in press), Zelcer et al. 1981). The presence or absence of these minor dsRNAs is a useful additional parameter which enhance the diagnostic value of this technique (Valverde et al. 1990). As these additional dsRNAs are usually less prominent, it was perhaps impossible to detect them on the electrophorogram of poor intensity obtained for dsRNA isolated from *D. surculosa*.

All these results of investigations suggest that the ringspot symptoms in *D. surculosa* may be provoked by a potyvirus infection, because infections with those viruses are widely spread in mono- and dicotyledonous plants (Milne 1988).

*

Acknowledgement. The authors are grateful to Dr. Mercedes Wrischer for micrographs published in this paper.

References

- Dodds, J. A., M. Bar-Joseph, 1983: Double-stranded RNA from plants infected with closteroviruses. *Phytopathology* 73, 419—423.
- Gildow, F. E., M. E. Ballinger, W. F. Rochow, 1983: Identification of double-stranded RNAs associated with barley yellow dwarf virus infection of oats. *Phytopathology* 73, 1570—1572.
- Krajačić, M., Z. Lorković, Optimizing alternative chromatographic approach in isolation of viral replicative dsRNA from infected plant tissue. *Acta Biologica HAZU* (in press).
- Lorković, Z., M. Krajačić, Double-stranded RNA from plants infected with two tymoviruses. *Acta Biologica HAZU* (in press).
- Milne, R. G., 1988: Taxonomy of the Rod-Shaped Filamentous Viruses. In: R. G. Milne (ed.), *The Plant Viruses*. Vol. 4, *The Filamentous Plant Viruses*, pp. 3—50. Plenum Press, New York and London.
- Pleše, N., M. Wrischer, 1978: Light and electron microscopy of cells infected with *Maclura* mosaic virus. *Acta Bot. Croat.* 37, 47—51.
- Valverde, R. A., J. A. Dodds, J. A. Heick, 1986: Double-stranded ribonucleic acid from plants infected with viruses having elongated particles and undivided genomes. *Phytopathology* 76, 459—465.
- Valverde, R. A., S. T. Nameth, R. L. Jordan, 1990: Analysis of double-stranded RNA for plant virus diagnosis. *Plant Disease* 74, 255—258.
- Zelcer, A., K. F. Weaver, E. Balazs, M. Zaitlin, 1981: The detection and characterization of viral-related double-stranded RNAs in tobacco mosaic virus-infected plants. *Virology*, 113, 417—427.

S A Ž E T A K

ETIOLOGIJA PRSTENASTE PJEGAVOSTI VRSTE *DRACAENA SURCULOSA* LINDL.

Mladen Krajačić i Nada Pleše

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Vrlo upadljivi simptomi prstenaste pjegavosti koji upućuju na virusnu infekciju, uočeni su na listovima biljke *Dracaena surculosa* Lindl. u Botaničkom vrtu Sveučilišta u Zagrebu. Pokušaji da se virusni uzroč-

nik prenese mehaničkom inokulacijom na niz standardnih pokusnih biljaka ostali su bez rezultata. Ipak, elektronskomikroskopskim pretragama ekstraktata alteriranih listova ustanovljena je nazočnost produženih virusnih čestica. U lisnom su soku također primijećene strukture koje bi mogle predstavljati dijelove virusnih staničnih uklopina »pinwheel« nazočnih, inače, pri infekciji potyvirusima. Dvolančana RNA, izolirana iz lisnog tkiva primjeraka sa simptomima, elektroforetskom pokretljivošću približno odgovara replikativnom obliku RNA potyvirusa. Stoga zaključujemo da bi simptomi prstenaste pjegavosti na vrsti *D. surculosa* mogli biti rezultat infekcije nekim potyvirusom.

Dr. Mladen Krajačić
Prof. dr. Nada Pleše
Botanički zavod
Prirodoslovno-matematički fakultet
Marulićev trg 20/II
41000 Zagreb, Hrvatska (Croatia)