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OCCURRENCE OF IVY VEIN CLEARING VIRUS IN YUGOSLAVIA

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English ivy (*Hedera helix* L.) plants with symptoms of vein clearing on the leaves have recently been found in the surroundings of Split (Croatia, Yugoslavia). These symptoms are characteristic of some diseases which are caused by rhabdoviruses. Therefore, we prepared ultrathin sections of leaves with vein clearing symptoms and we found rhabdoviruses in these leaves. The rhabdovirus particles were located in the cytoplasm. They were 60 to 70 nm wide but the length of virus varied greatly. Afterwards, we succeeded in transmitting this virus mechanically by means of corresponding buffer solution and sodium ascorbate to a suitable test plant.

According to these data we could conclude that the rhabdovirus found is ivy vein clearing virus which has been first detected and described in Italy. Yugoslavia is the second state in which this rhabdovirus has been found.

Introduction

In the early spring 1982 we found specimens of English ivy (*Hedera helix* L.) with obvious yellowing of lateral leaf veins in the form of a net (Fig. 1 A). Leaf symptoms were similar to those described first by Russo et al. (1979) on English ivy in Southern Italy. In diseased plants these authors found a rhabdovirus which matured and accumulated in the cytoplasm.

Already Russo et al. (1979) gave this virus the name of ivy vein clearing virus (IVCV). They also established that this virus had bullet-shaped particles which were 60 to 70 nm wide. On the surface of the

particles a large number of projections, about 8 nm long, were situated. The coiled nucleocapsid had a large number of transverse striations, which were 5 nm distant from one another. The length of IVCV was later determined by Castellano and Rana (1981), who found that the particles were mostly 100 to 200 nm long, sometimes from 250 to 300 nm.

Castellano and Rana (1981) succeeded in transmitting IVCV mechanically from infected English ivy to herbaceous plants *Gomphrena globosa L.* and *Nicotiana benthamiana* Domin. Later on, using these test plants, they transmitted IVCV mechanically to some other *Nicotiana* species and *Datura stramonium* L. The infected *Nicotiana* helped them to investigate the stability of IVCV in plant sap.

As we observed English ivy leaves with vein yellowing symptoms in Yugoslavia, we decided to begin this investigation.

Material and Methods

Material for this investigation was collected in a garden in the surroundings of Split. The English ivy plant had well developed symptoms of yellow netting on some leaves (Fig. 1 A). In many parts of the town similar symptoms on English ivy were fairly frequent. The symptoms were often restricted to one part of the leaf blade only and sometimes only to some leaves of a twig. The leaf symptoms were often accompanied by other malformations such as curving and distortions.

Attempts to isolate the virus in the warmer season remained unsuccessful.

Young leaves of naturally infected English ivy with symptoms of vein yellowing on lateral veins were taken for electron microscopy. Small pieces of leaves were fixed in 1% (v/v) glutaraldehyde in cacodylate buffer for 30 min and then postfixed in OsO_4 for 2 h. The samples were dehydrated in alcohol series and embedded in Epon 812. The material was subsequently cut with a diamond knife. Ultrathin sections were stained with uranyl magnesium acetate (Kimura et al. 1975) and lead citrate. Finally, the sections were examined in a JEM 100 B electron microscope.

For mechanical transmission of IVCV, young leaves of naturally infected English ivy with initial symptoms of vein yellowing were used. The material was homogenized in cold neutral 0.1 M phosphate buffer containing 1% sodium ascorbate. For isolation of IVCV the common test plants were used which are susceptible to plant rhabdoviruses.

Results

During a short visit to Croatia in spring 1981 Dr. G. L. Rana (Bari, Italy) drew our attention to the presence of vein yellowing symptoms on English ivy in the surroundings of Split. The next year we collected naturally infected ivy plants on this location and prepared the first electron microscopic figures of rhadboviruses (Fig. 1).

The particles were found in the parenchyma cells of leaf region where the secondary veins were developed. There they were observed in the cisternae of endoplasmic reticulum. In Fig. 1 B and C two cisternae are presented in which the particles are sectioned longitudi-

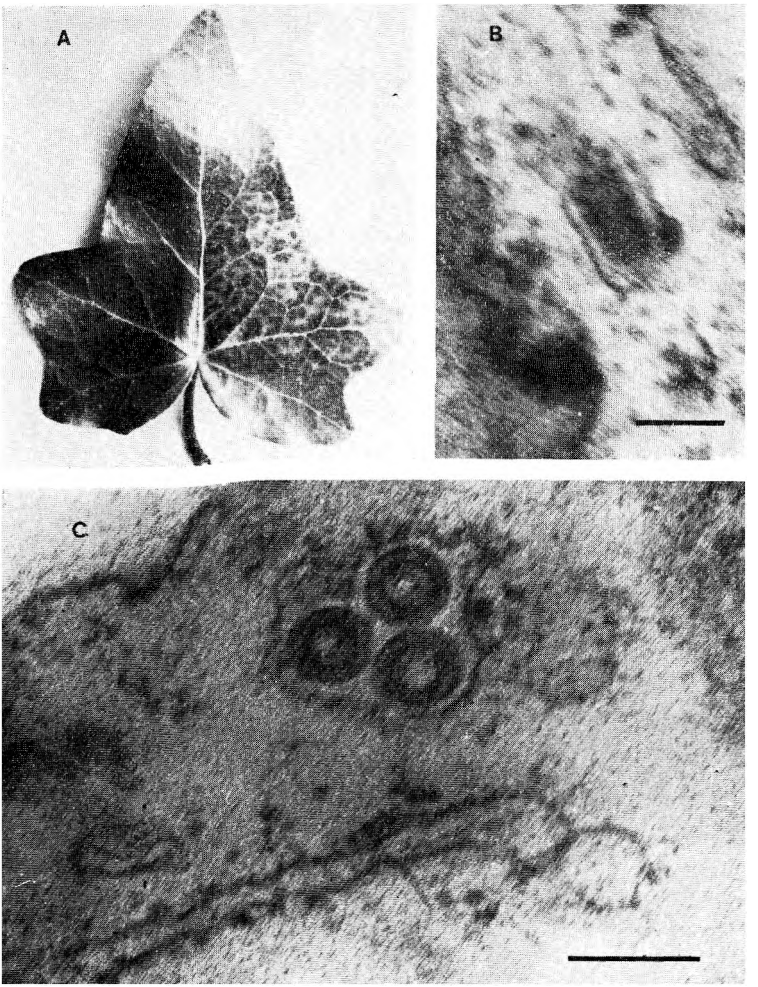

Fig. 1. A. On a part of ivy leaf, intensive chlorosis of small veins is visible. B. In an elongated cisterna a longitudinally sectioned rhabdovirus particle is situated. C. An enlarged cisterna of endoplasmic reticulum with three transversally sectioned virus particles. — Bars represent 100 nm.

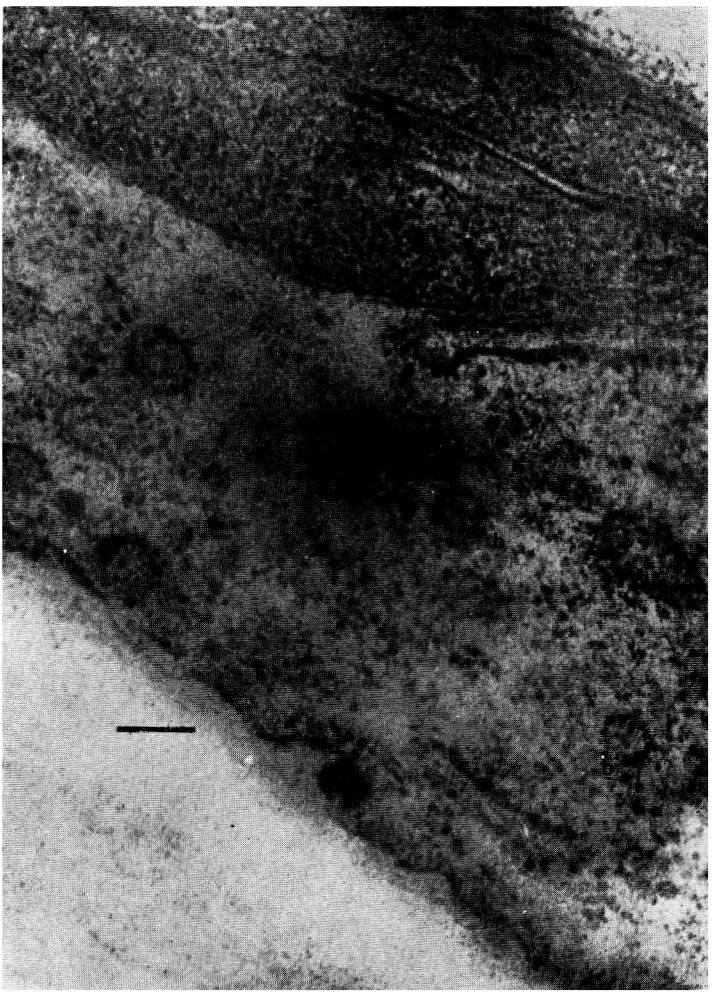

Fig. 2. In the cytoplasm some circular bodies (b) similar to the »electron opaque bodies« of Russo et al. (1979) are present. — Bar represents 100 nm.

nally and transversally. In Fig. 1 C the internal structure of virus particles is visible. The dimensions of the particles correspond well with the data reported by Russo et al. (1969) and by Castellano and Rana (1981). During the survey of ultrathin sections we did not see virus particles near the nucleus, nor in the perinuclear cisternae. Consequently, the particles of this rhabdovirus are localized in the cytoplasm and they avoid the region on of nucleus.

During the survey of electron microscopic sections we found, in addition to rhabdoviruses, some »circular electron opaque bodies« with a diameter of about 55 nm. These bodies have been well described and illustrated by Russo et al. (1979).

As Castellano and Rana (1981) succeeded in transmitting IVCV to herbaceous plants, we tried also to perform similar experiments. Therefore, we prepared an inoculum in the manner described. As test plants were used *Gomphrena globosa* L., *Nicotiana tabacum* L. White Burley and *N. glutinosa* L. The first symptoms appeared after twenty days p. i. on the specimens of *G. globosa*. We observed local symptoms which consisted of dark red rings with a whitish centre. Similar symptoms were observed by Castellano and Rana (1981) also on *G. globosa*. Consequently, we consider that rhabdovirus IVCV is spread in the seaside region of Yugoslavia.

Discussion

On the basis of these data it seems that IVCV is not spread only in Italy but also in Yugoslavia. According to our actual knowledge IVCV is the second rhabdovirus found in Yugoslavia which matures and accumulates in the cytoplasm.

The first rhadbovirus discovered in Yugoslavia was pittosporum vein clearing virus (PVCV) found in *Pittosporum tobira*. It was discovered in the seaside part of the country. Later it was thoroughly investigated by Plavšić-Banjac et al. (1976), Plavšić et al. (1976), Plavšić et al. (1978), Rana and Di Franco (1979) and Marani and Bertaccini (1980). The most important finding was made by Rana and Di Franco (1979) who have established that PVCV is transmissible mechanically to herbaceous test plants. In these plants PVCV was throughly investigated.

Another rhabdovirus found in Yugoslavia was laburnum vein clearing virus (LVCV). It was first described by Schultz and Harrap (1975) on *Laburnum anagyroides* in England. Afterwards, it was found in some places in Zagreb in the same plant (Plešе 1979). Later ons, Miličić (1982) established, again in Zagreb, the presence of this virus on *Laburnum anagyroides* and *Laburnum alpinum* in the Botanical Gardens of the Faculty of Science in Zagreb.

The next finding of rhabdoviruses concerns the plant *Euonymus japonica*. The virus was described by Codaccioni and Cossard (1975, 1977) who succeeded in transmitting the virus by grafting to nonfasciated exemplars of *E. japonica* but the infested plants retained the normal form, i.e. they did not become fasciated. This virus is registered in the literature as euonymus fasciation virus (Matthews 1982).

Later Plešе and Erić (1980) investigated a rhabdovirus found in a nonfasciated exemplar of *E. japonica*, which was collected in Paris (France) and then studied in Zagreb. In this shrub they detected a rhabdovirus which was 300 nm long and 70 nm wide.

In the same paper Pleše and Erić (1980) described another rhabdovirus from *Euonymus japonica* var. *microphylla* which was cultivated in the Botanical Gardens in Zagreb. This shrub contained also a rhabdovirus which budded on the inner perinuclear membrane and had similar dimensions as the virus from France. It seems that these two *Euonymus* viruses are similar to, or identical with the rhabdovirus first described by Codaccioni (1972).

In Yugoslavia, another rhabdovirus is also spread, which is interesting for horticulture. This virus is raspberry vein chlorosis virus (Jordović 1963, Jones et al. 1977). The rhabdovirus is transmitted by the aphid *Aphis idaei* and it is wide-spread throughout the world. The virus matures in the cytoplasm and is 65 × 430 nm large (Francki et al. 1981).

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SAŽETAK

NALAZ VIRUSA ŽUĆENJA ŽILA BRŠLJANA U JUGOSLAVIJI

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Primjerci bršljana (*Hedera helix L.*) sa simptomima prosvjetljavanja lisnih žila nađeni su nedavno u okolici Splita. Ti su simptomi karakteristični za neke bolesti koje izazivaju rabdovirusi. Zbog toga smo priredili ultratanke presjeke kroz listove s prosvjetljavanjem žila i pronašli smo rabdoviruse u tim listovima. Čestice rabdovirusa nalazile su se u citoplazmi. Bile su 60 do 70 nm široke, ali je dužina virusa bila vrlo različita. Poslije toga uspjeli smo prenijeti taj virus mehanički pomoću odgovarajuće puferske otopine i natrijeva askorbata na pogodnu pokusnu biljku.

Na osnovi tih podataka mogli smo zaključiti da nađeni rabdovirus pripada virusu prosvjetljavanja žila bršljana (ivy vein clearing virus) koji je najprije nađen u Italiji. Jugoslavija je druga zemlja u kojoj je taj virus otkriven.

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