DETECTION AND ISOLATION OF NEPOVIRUSES ON STRAWBERRY IN THE CZECH REPUBLIC

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Summary. – Arabis mosaic, strawberry latent ringspot, tomato black ring and raspberry ringspot nepoviruses were monitored using double sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in 18 cultivars of strawberry *Fragaria* x *ananassa Duch.* in the Czech Republic. Arabis mosaic and strawberry latent ringspot viruses were detected, isolated and characterized on differential host plants and by electron microscopy. Both viruses were purified and antisera to them were prepared.

Key words: nepoviruses; strawberry; detection; purification

Introduction

Meristem tip culture derived and *in vitro* multiplied seedlings of strawberry were introduced in horticulture practice in the Czech Republic during eighties. However, with the exception of strawberry latent ringspot virus (SLRV, Novák and Lanzová, 1979), there is no information on the occurence of nepoviruses on strawberries in this territory. Therefore our investigation was aimed at the detection, isolation and characterization of isolates of arabis mosaic (AMV), SLRV, raspberry ringspot (RRV) and tomato black ring (TBRV) viruses (nepoviruses), which were reported to be of importance in cultivated strawberries in Europe by Murant and Lister (1987).

Materials and Methods

Collection of samples. Four hundred and seventy-one samples of cultivars Adriana, Bounty, Dagmar, Dukát, Elista, Elsanta, Elvíra, Gorella, Induka, Kama, Karmen, Korona, Lidka, Ostara, Redgauntlet, Rujana, Senga Sengana and Zefýr were collected in 2 – 3 year-old strawberry plantations in Southern (Planá near České Budějovice, Lhenice, Třísov, Křemže), Central (Jesenice near Prague) and Eastern Bohemia (Breeding Station Turnov) from 1991 to 1993.

DAS-ELISA. Nepoviruses were detected by DAS-ELISA (Clark and Adams, 1977) using diagnostic kits (Loewe Biochemica) with alkaline phosphatase-conjugated antibodies. Samples with absorbancy (A) higher than $\bar{x}$ + 3 SD ($\bar{x}$ - mean value of the non-infected controls, SD = standard deviation) were scored as positive.

Immunosorbent electron microscopy (ISEM). Immunoglobulins (IgG) from diagnostic kits were diluted 1:1000 in 0.1 mol/l sodium/potassium phosphate buffer pH 7.0. Grids were floated for 5 mins on diluted IgG, rinsed with 40 drops of the phosphate buffer, transferred to leaf homogenate of DAS-ELISA positive samples and incubated at 20 °C overnight. Grids were rinsed with 50 drops of distilled water before staining with 2% uranyl acetate.

Inoculation of test plants. For the homogenization of DAS-ELISA positive samples, four inoculation buffers were used: 1% (w/v) polyethylene glycol in 0.066 mol/l phosphate buffer pH 7.0; 0.066 mol/l phosphate buffer pH 7.0; 2% (v/v) nicotine in distilled water, and 0.005 mol/l borate buffer pH 8.6. Differential host plants (Table 1) were me-

Abbreviations: AMV = arabis mosaic virus; DAS-ELISA = double sandwich enzyme-linked immunosorbent assay; IgG = immunoglobulin; ISEM = immunosorbent electron microscopy; RRV = raspberry ringspot virus; SLRV = strawberry latent ringspot virus; TBRV = tomato black ring virus

chanically inoculated and their reaction was evaluated 21 days after inoculation.

Purification and electron microscopy. The AMV and SLRV isolates were propagated in *Chenopodium quinoa* Willd. and in *Cucumis sativus* L., respectively. Infected plants were harvested 21 days after inoculation and the viruses were purified by a modified method of Savino *et al.* (1979). Infected leaves were homogenized in a blender with 0.1 mol/l phosphate buffer pH 7.0 containing 0.01% thioglycolic acid. The homogenate was filtered and the sap clarified by stirring with 6% suspension of Mg-activated bentonite (Dunn and Hitchborn, 1965). The supernatant was subjected to two cycles of differential centrifugation (2 hrs at 29 000 rpm, 10 mins at 12 000 rpm, 1 hr at 50 000 rpm, 10 mins at 12 000 rpm). Final pellets were resuspended in 0.02 mol/l phosphate buffer pH 7.2.

Further purification was conducted by centrifugation in 10 – 40% sucrose density gradient in 0.02 mol/l phosphate buffer pH 7.2 for 4 hrs at 24 000 rpm in SW 28 rotor (Beckman). Purificates were analysed in UV spectrophotometer (Pharmacia). Purified virions were stained with 2% uranyl acetate and examined in Jeol 100 MB electron microscope.

Preparation of antibodies. Antisera against AMV and SLRV isolates were prepared each in two rabbits immunized by one subcutaneous and one intramuscular injection of the virus emulsified in Al-Span Oil incomplete adjuvant, followed by 3 intravenous injections given in 7 days intervals. For each injection 0.5 mg of purified virus was used.

Titer of prepared antisera was determined by double diffusion in 0.7% Difco Noble agar in 0.018 mol/l McIlvaine buffer pH 7.0 containing 0.02% sodium azide. Leaves of *Ch. quinoa* and *C. sativus* with systemic infection by AMV and SLRV served as antigens.

Results and Discussion

AMV

Two plants of cv. Kama with typical prominent blotching symptoms of "strawberry mosaic" (Posnette, 1956) (A_{405} = 0.402) and cv. Redgauntlet with symptoms of leaf deformation (A_{405} = 0.349) from Planá near České Budějovice were AMV-positive in DAS-ELISA. Mean A_{405} values of healthy and positive controls were 0.008 and 0.563, respectively. SLRV, RRV or TBRV were not detected in these samples.

The virus was transmitted from cv. Kama only to test plants (Table 1). The symptoms on diffrential plants were similar to those described for English (Harrison, 1958) and German (Lister and Krczal, 1962) isolates of AMV from strawberries, excepting *Petunia hybrida, Nicotiana rustica,*

Table 1. Reaction of herbaceous test plants after mechanical inoculation with Czech isolates of AMV and SLRV

Host plant	Virus			
	AMV		SLRV	
	I	II	I	II
Chenopodium quinoa Willd.	NS	M,D,C	(CL)	M,C,N
C. amaranticolor Coste et Reyn.	NS	M,D,C	NS	D,M
C. album L.	NS	M,D	(CL)	M,D
C. murale L.	NS	M,D	NS	M,D
Cucumis sativum L.	(CL)	M,S,St	(CL)	IC
Nicotiana tabacum L. sv. Samsun	NS	NS	NS	NS
N. tabacum cv. Xanthi	NS	NS	NS	NS
N. tabacum cv. White Burley	NS	NS	NS	NS
N. glutinosa L.	NS	NS	NS	NS
N. rustica L.	NS	NS	NS	NS
Petunia hybrida Hort ex Vilm.	NS	NS	NS	NS
Phaseolus vulgaris L. cv. Blanka	NS	M,N,D	NS	M
Physalis floridana Rybd.	NS	NS	NS	NS

C – collapse od young leaves
CL – chlorotic lesions
D – distortion of young leaves
IC – interveinal chlorosis
M – mosaic
I – reaction on inoculated leaves
N – necroses
NS – no symptoms
S – yellow spotting
St – stunting
( ) – sporadic case
II – systemic infection

N. glutinosa and *N. tabacum* cv. White Burley, which were not found to be symptomatic hosts for the Czech isolate. The latter did not produce primary local lesions in *Chenopodiaceae* plants unlike isolates described previously by Harrison (1958) and Lister and Krczal (1962).

From 350 g of infected tissue of *C. guinoa* 7 mg of virus were purified ($A_{260/280}$ = 1.46). The purified virus showed UV absorption spectrum characteristic for nucleoprotein with maximum between 257.7 and 260.0 nm and minimum between 243.9 and 249.6 nm. In sucrose gradients the virus exhibited three peaks which corresponded to the bottom, top an intermediate components of AMV-M.L. and AMV-M.H. isolates reported by Bellardi *et al.* (1986).

Electron micrographs of purified isolate showed "full" nucleoprotein particles and "empty shells" (capsids only) about 28 nm in diameter (Fig. 1) similarly to the results of Bellardi *et al.* (1986) and Murant (1970). Homologous titer of the antiserum was 1:512.

SLRV

SLRV was isolated from cv. Induka with symptoms of severe leaf mosaic, stunting and reduced yield collected at Planá near České Budějovice. A_{405} value for SLRV ELISA was 1.3, and mean values for healthy and positive controls were 0.001 and 1.606, respectively. AMV, TBRV and RRV were not detected in this sample. Reactions of differential host plants are summarized in Table 1. The symptoms were similar to those of Hampshire strawberry isolate of SLRV, with the exception of *C. amaranticolor* and *Phaseolus*

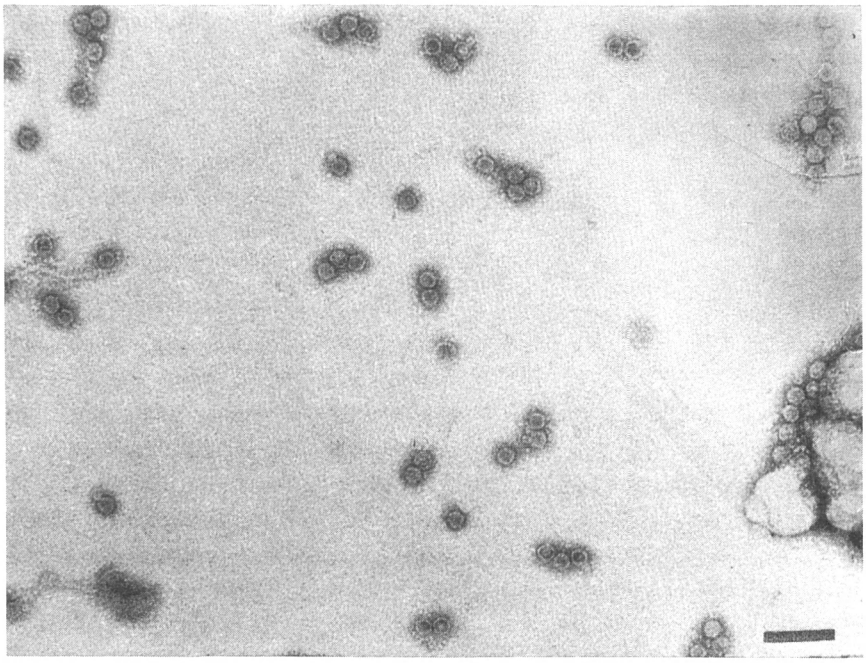

Fig. 1
Electron micrograph of purified Czech isolate of AMV from strawberry cv. Kama (bar = 100 nm)

vulgaris, which did not produce chlorotic primary lesions found by Lister (1964).

Electron micrographs from partially purified preparations showed numerous particles about 28 nm in diameter (Fig. 2) similarly to the results of Murant (1974) and Credi *et al.* (1981).

From 700 g of infected plant material 7.7 mg of non-separated virus were obtained ($A_{260/280}$ = 1.5). Because the partially purified preparations were suficiently pure, further purification by centrifugation in sucrose density gradients was not conducted.

The antiserum had a homologous titer of 1:256 and reacted very weakly with the sap from healthy plants of *Cucumis sativus* in a dilution of 1:1 only.

TBRV and RRV

Four symptomless samples (cv. Gorella I/1, A_{405} = 0.372; Gorella I/3, A_{405} = 0.437; Gorella II/6, A_{405} = 0.343; Senga Sengana VI/7, A_{405} = 0.539) from Breading Station Turnov were positive in DAS-ELISA for TBRV. Mean A_{405} values of healthy and positive controls were -0.006 and 0.138, respectively.

Thirty-eight strawberry plants from all localities except Křemže were positive in DAS-ELISA for RRV. A_{405} values of positive samples varied from 0.400 to 0.958. Mean values of healthy and positive controls were 0.015 and 0.889, respectively.

Although TBRV and RRV were described as readily transmissible by mechanical inoculation (Murant, 1970a, 1978), we inoculated about 2 000 differential host plants using all four inoculation buffers without any success. Moreover, no virus particles were trapped from sap extracts of DAS-ELISA-positive samples by ISEM.

Novák and Lanzová (1979) isolated and transmitted to differential host plants two virus isolates from strawberries in the Czech Republic which were identified as SLRV in double diffusion test. Our results confirmed the occurrence of SLRV and clearly demonstrated the presence of AMV on strawberry in the Czech Republic. Although A_{405} values of 4 samples (0.85%) in DAS-ELISA for TBRV and of 38 samples (8.07%) for RRV out of 471 samples tested were relatively high, we failed in detecting these viruses by other methods. Therefore their presence in strawberry in the Czech Republic remains dubious.

All four nepoviruses were found to occur in strawberry in England, Lister (1958, 1960, 1960a, 1964) and Russia (Keldysh *et al.*, cit. Murant and Lister, 1987). AMV and SLRV were found in Hungary (Szilágyi, 1980), AMV in Germany (Lister an Krczal, 1962) and Irish Republic (Staunthnon and Moore, 1967). In contrast to severe outbreaks of nepoviruses in strawberry reported by Lister (1958), Lister and Krczal (1962), and Murant and Lister (1987), we detected AMV in only two plants (0.42%) and SLRV in one plant (0.21%) of 471 plants tested, although samples were taken predominantly from those showing

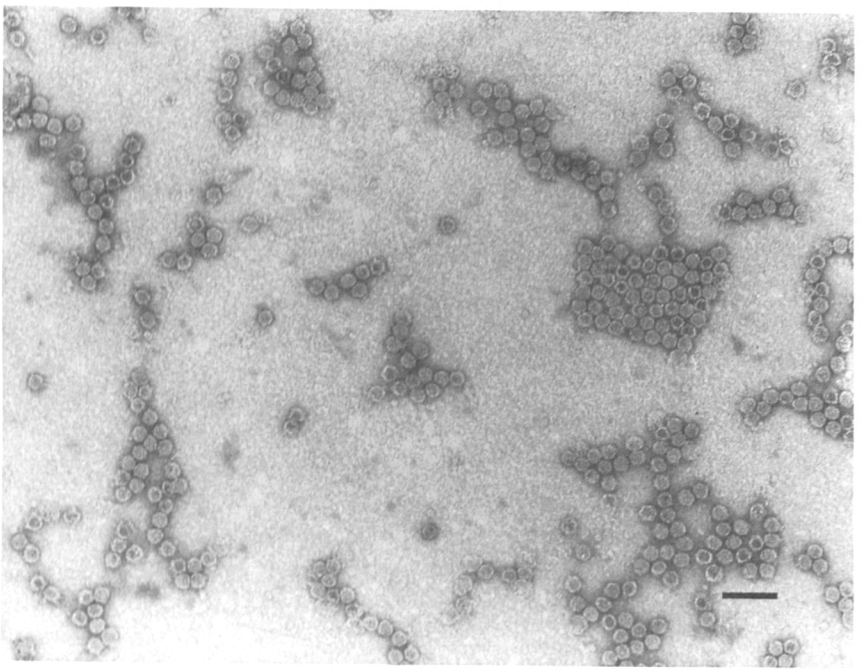

Fig. 2
Electron micrograph of purified Czech isolate of SLRV from strawberry cv. Induka (bar = 100 nm)

symptoms. Our investigation indicates low incidence of nepoviruses in strawberry in the Czech Republic, which may be a positive consequence of long-term exploitation of meristem-derived seedlings by growers.

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