OCCURRENCE OF NEPOVIRUSES IN *RUBUS* SPECIES IN THE CZECH REPUBLIC

J. ŠPAK, D. KUBELKOVÁ, J. HONETŠLEGROVÁ-FRÁNOVÁ

Department of Plant Virology, Institute of Plant Molecular Biology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

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Summary. – The occurrence of arabis mosaic virus (AMV), raspberry ringspot virus (RRV), tomato black ring virus (TBRV), strawberry latent ringspot virus (SLRV) and cherry leaf roll virus (CLRV) in cultivated and wild plants of raspberry and blackberry has been studied in the Czech Republic in 1993 – 1996. Five hundred and seventy samples were collected at 51 localities and assayed by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The results represent the first evidence on the occurrence of AMV, RRV, TBRV and SLRV in cultivated *Rubus* species in the Czech Republic. Isolates AMV M20 and TBRV ML15 which were successfully transmitted by mechanical inoculation and characterized by reactions of differential host plants and by electron microscopy are the first isolates from *Rubus* from this territory. CLRV was not detected in either cultivated or wild *Rubus* species.

Key words: nepoviruses; Czech Republic; raspberry; blackberry; ELISA

Introduction

Nepoviruses (genus *Nepovirus*, family *Comoviridae*) are distributed in *Rubus* sp. world-wide (Jones, 1986). There are a few early notes on the occurrence of virus diseases of *Rubus* sp. in former Czechoslovakia, but most of them only described symptoms on infected plants (Blattný, 1927; Novák, 1950; Helebrant, 1958). Since 1984 a multiplication scheme for virus-free raspberry and blackberry seedlings has been initiated (Janečková, 1987; Janečková and Pluhař, 1987), but any data on the occurrence and epidemiology of viruses in *Rubus* were missing. The first, and so far the only serological evidence of viruses in *Rubus* was published by Pelikán and Smrčka (1988) and Smrčka (1990, 1993) who reported AMV, TBRV, SLRV, CLRV and CMV in spontaneously infected wild raspberries. This prompted us to conduct a study on nepoviruses in *Rubus* with emphasis on cultivars and seedlings produced in the Czech Republic.

Abbreviations: AMV = arabis mosaic virus; CLRV = cherry leaf roll virus; CMV = cucumber mosaic virus; DAS = double antibody sandwich; ELISA = enzyme-linked immunosorbent assay; RRV = raspberry ringspot virus; SLRV = strawberry latent ringspot virus; TBRV = tomato black ring virus

Materials and Methods

Collection of samples was carried out during 1993 – 1996 from raspberry and blackberry plantations and canopies of wild raspberries and blackberries with symptoms on leaves in 51 localities in Southern, Central and Eastern Bohemia. Samples of 19 cultivars taken from the nursery at Breeding Station in Velké Losiny (Northern Moravia) were symptomless.

ELISA. Samples were tested by DAS-ELISA with diagnostic kits according to Clark and Adams (1977) following the manufacturer's protocols. AMV, TBRV, SLRV and RRV were assayed with kits purchased from Loewe Biochemica (Germany). For CLRV Sanofi (France) and Loewe Biochemica (birch isolate, and elderberry isolate) kits were used. Samples with A_{405} higher than $x + 3SD$, (x = value of healthy control, SD = standard deviation) were scored as positive (Sutula *et al.*, 1986).

Virus isolation. Samples positive in ELISA were homogenized in 2% nicotine solution in water and mechanically inoculated onto differential host plants (Table 3) using carborundum powder as abrasive. Twenty plants of each indicator were used per test and reactions of host plants were evaluated during 3 weeks after inoculation. Plants were kept in an insectproof greenhouse and positive infections were confirmed by ELISA and electron microscopy.

Electron microscopy. Samples for electron microscopy were prepared from leaves of differential host plants with symptoms of infection. Formwar- and carbon-coated grids were floated on drops of sap, stained with 2% uranyl acetate and examined in Jeol 100 MB electron microscope.

Results and Discussion

Samples of cultivated (401) and wild raspberries (125), as well as of cultivated (12) and wild blackberries (29) from 51 localities were tested by DAS-ELISA. The samples showed symptoms ranging from mild green or vein mosaic to distinct yellow mosaic and leaf deformation (Table 1). There was a distinct difference in virus occurrence in cultivars grown in commercial plantations, in cultivated or semi-cultivated plants in home gardens and in wild plants in forests.

One hundred and twenty-four symptomless samples of 19 raspberry and 2 blackberry cultivars derived from meristem tip cultures at the Research and Breeding Institute of Fruit Crops, Holovousy, Czech Republic, used for propagation at the Breeding Station, Velké Losiny, Czech Republic, were found negative for the assayed viruses in 1995 and in repeated tests in 1996. Similarly, we found only 4 TBRV- and 2 RRV-positive plants among 22 plants with virus-like symptoms collected from a raspberry plantation at Mnichovo Hradiště, while in other plantations at Jesenice and Lhenice (all in Czech Republic) we found no ELISA-positive samples. This may represent a positive result of the virus eradication programme set up in 1985 (Janečková, 1987).

Higher incidence of nepoviruses in cultivated or semicultivated raspberries only exceptionally of known origin grown for tens of years on a same place was not surprising. Twenty-eight (18.7%) of 125 samples were found to be infected at least with one nepovirus, and 5 of them with two nepoviruses in a mixed infection (Table 2). RRV was the most abundant virus infecting 50% of positive samples. AMV, RRV, TBRV and SLRV were detected in raspberries, while in blackberries only RRV and SLRV were found.

AMV M20 isolate from Třtěnice (East Bohemia) was successfully transmitted from wild raspberry with symptoms of yellow chlorotic spots. TBRV ML15 isolate was obtained from wild raspberry with chlorotic spots on leaves near strawberry field in Lhenice (South Bohemia). The reactions of differential hosts are summarized in Table 3. Electron microscopy of isolates AMV M20 and TBRV ML15 revealed spherical virions 28-30 nm in diameter in sap from leaves of infected host plants. Nepoviruses were described as readily transmissible by mechanical inoculation (Jones, 1986), but we achieved only a low transmissibility as in previous experiments on the isolation of nepoviruses from strawberries (Honetšlegrová and Špak, 1995). Although we inoculated all samples positive in ELISA onto hundreds of differential host plants, using *Chenopodium quinoa* of Dutch (IPO, Wageningen) and Scottish (SCRI, Dundee) prove-

Table 1. Occurrence of RRV, AMV, TBRV, SLRV and CLRV in 570 ELISA-assayed *Rubus* plants from different localities in the Czech Republic

Virus	Host plant				
	Raspberry			Blackberry	
	Plantations	Gardens	Wild	Gardens	Wild
RRV	2	14	8	0	3
AMV	0	4	1	0	0
TBRV	4	5	2	0	0
SLRV	0	5	1	0	1
CLRV	0	0	0	0	0
Total	276	125	128	12	29
% of infected plants	2.2	22.4	9.4	0.0	13.8

Table 2. Samples of *Rubus* plants with mixed infection with nepoviruses

Isolate	Locality	Host plant	Symptoms	Viruses
B I	Bošilec	raspberry cultivar	vein mosaic, leaf deformation	AMV+RRV
B II	Bošilec	raspberry cultivar	vein mosaic, leaf deformation	AMV+SLRV
L 5	Lhenice	wild raspberry	vein mosaic	TBRV+SLRV
J 11	Jesenice	wild raspberry	vein mosaic	AMV+RRV
St	Jičín	wild blackberry	mosaic	RRV+SLRV

Table 3. Reaction of differential host plants to inoculation with AMV and TBRV isolates

Host plant	Isolate			
	AMV M20		BRV ML15	
	I	II	I	II
Cucumis sativus L. cv. Bílská	LCL	MO	–	CM
Petunia hybrida Hort. cv. Lavina	LNR	NR	–	–
Chenopodium quinoa Wild.	LCL	CL	LNL	CL
Ch. amaranticolor Coste et Reyn.	–	–	LNL	CL
Phaseolus vulgaris L. cv. Blanka	–	M	–	–
Nicotiana tabacum L. cv. Xanthi	–	–	LNR	NP
Nicotiana tabacum L. cv. Samsun	–	–	–	NR
Nicotiana rustica L.	–	–	–	NP

I = symptoms on inoculated leaves; II = systemic symptoms; LCL = local chlorotic lesions; LNR = local necrotic rings; LNL = local necrotic lesions; MO = mottling; M = mosaic; CM = chlorotic mosaic; NR = necrotic rings; NP = necrotic patterns; CL = chlorotic lesions; CR = chlorotic rings.

nience and different inoculation buffers, we had only a little success with their transmission.

Smrčka (1990) found 34% of wild *Rubus* symptom-positive plants infected with AMV or TBRV using DAS-ELISA with horseradish peroxidase-labelled immunoglobulins G. Later, Smrčka (1993) found 66% of wild *Rubus idaeus* plants infected with AMV, TBRV, SLRV and CMV near Prague but no data about the origin or quality of antibodies used in this test are mentioned in both publications. In contrast to his results only 9.4% of infected wild plants of raspberry found in our tests seems to be quite low. This discrepancy may be explained by considering only mosaic symptoms on leaves by Smrčka in contrast to all types of symptoms considered by us. Besides, samples of Smrčka originated mostly from warmer lowlands of Central Bohemia.

We found no correlation between the presence of certain virus and the symptoms of infection. This is not surprising as the samples were tested only for the presence of five nepoviruses. Further nepoviruses and aphid-borne viruses known to occur in *Rubus* (Jones, 1986) could be present in these plants but no data are available on their occurrence in the Czech Republic so far.

Pelikán and Smrčka (1988) have published a short note about the occurrence of CLRV in wild raspberries in South Bohemia proved by double diffusion test in agar. Unfortunately, they did not mention the origin and quality of the antiserum, number of infected plants and efforts to isolate the virus. To verify this unique finding registered by EPPO (Anonym, 1991), we searched for this virus in *Rubus* plants in all localities including surroundings of the town of České Budějovice where the virus was found. Although we used 3 commercially available kits based on IgG against different CLRV isolates we found no positive plants. Therefore we could not confirm the occurrence of CLRV in cultivated or wild *Rubus* plants in the Czech Republic.

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