QUARANTINE STRAWBERRY VEIN BANDING VIRUS FIRSTLY DETECTED IN SLOVAKIA AND SERBIA

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Summary. – Strawberry vein banding virus (SVBV) was detected by polymerase chain reaction (PCR) and dot-blot hybridisation in samples of cultivated strawberry plants originating from central Slovakia and in samples of wild strawberry plants from south-eastern Serbia in Federal Republic of Yugoslavia (FRY). This is the first finding of SVBV in these countries as well as of SVBV in wild strawberry plants in Europe.

Key words: strawberry vein banding virus, caulimovirus; strawberry; virus detection; polymerase chain reaction; dot-blot hybridisation

Introduction

Strawberry vein banding (SVB) disease was described firstly in the USA in 1955 (Frazier, 1955). Reports on its occurrence in Europe before 1995, when the isolation and nucleic acid-based detection of SVBV (genus *Caulimovirus*) was published (Honetšlegrová *et al.*, 1995), have been based exclusively on the disease symptoms and are, therefore, of limited value only.

SVBV alone does not produce clear symptoms even on „susceptible" strawberry clones and its biological detection is unreliable. SVB symptoms are more expressed provided SVBV occurs in mixed infection with other strawberry viruses (Bolton 1974; Freeman and Mellor, 1962). It seems that most of typical SVB symptoms are caused by these mixed infections rather than by SVBV infection alone. Because of supposed low incidence, this disease is of minor importance. Nevertheless, SVBV was placed among quarantine plant viruses monitored in the EU (Smith *et al.*, 1997).

Reliable and sensitive nucleic acid-based detection methods for SVBV were developed (Mráz *et al.*, 1996a, 1997) and complete sequence of circular dsDNA genome of SVBV was elucidated recently (Petrzik *et al.*, 1998). This progress allowed us to confirm the occurrence of SVBV in plant samples from the USA and in cultivated strawberries from Germany and Norway (Mráz *et al.*, 1996b). Several SVBV isolates were described in the Czech Republic (Honetšlegrová *et al.*, 1995). Two of them differed in the coat protein sequence from isolates from the USA and western Europe (Mráz *et al.*, 1998).

In screening the SVBV spread in some central and south European countries we detected the virus in Slovakia and in Serbia, the FRY.

Abbreviations: FRY = Federal Republic of Yugoslavia; nt = nucleotide; PCR = polymerase chain reaction; SVB = strawberry vein banding; SVBV = strawberry vein banding virus

Materials and Methods

Plant samples. Samples of strawberry cultivars Mária, Senga Sengana and Magura originated from plants growing on field in central Slovakia (200 km NE of Bratislava). Samples from Serbia, the FRY, were randomly selected from various cultivars and wild *F. vesca* strawberry from Kukavica Mountain (250 km SE of Belgrade).

PCR. DNA was isolated from 0.6 cm disks of leaf tissue by the alkaline lysis method as described elsewhere (Trněná *et al.*, 1995). PCR was performed with two primer pairs. Primers I2 and I3 were derived from the SVBV coat protein gene and produced an ampli-

fied fragment of 603 bp (Mráz *et al.*, 1997). Primers C8 (upstream, 5'-TCTTTCAGAATATGAAGCCG-3') and C9 (downstream, 5'-GTTCGCCTCTGCCTTCAAATG-3') located in the large intergenic region (nt 6887–7241) amplified a product of 355 bp. The reaction conditions were the same as described previously with the exception that C8 and C9 primers were annealed at 50°C.

Dot-blot hybridisation. A DIG-labelled probe was prepared from the I2–I3-primed PCR product from pSVBV-E3 plasmid containing the full-length clone of SVBV (Mráz *et al.*, 1996a). Five µl of the PCR mixture after amplification was spotted on a nylon membrane and hybridised (Mráz *et al.*, 1997).

Results and Discussion

We tested 75 plant samples from Serbia. All but one sample (No. 16) did not produce a visible PCR amplification product with I2–I3 primers after electrophoretic separation (Fig. 1). In parallel, aliquots of the PCR mixtures were subjected to dot blot hybridisation with the SVBV-specific probe. Only the sample No. 16 that was PCR-positive produced a visible spot on the blot (Fig. 2).

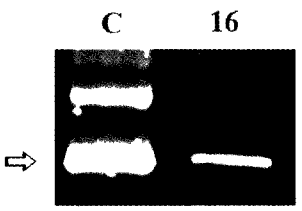

Fig. 1
Detection of SVBV in the sample No. 16 from Serbia by PCR and agarose gel electrophoresis
pSVBV-E3 positive control (lane C). Arrow marks the position of SVBV-specific PCR product of 603 bp.

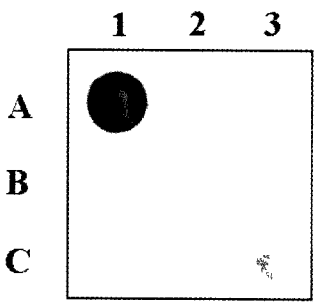

Fig. 2
Dot-blot hybridisation of the samples from Serbia
pSVBV-E3 positive control (1A), healthy controls Alpine (1B) and VC1 (1C), the samples (2A–3C). Sample No. 16 is located at 3C.

Plant samples from Slovakia were deliberately frozen-thawed several times before analysis and their nucleic acid was supposedly seriously damaged. Therefore we used another primer pair (C8–C9) which produced a smaller amplification product of 355 bp with pSVBV-E3 plasmid as a template (Fig. 3, lane C). The probability of the presence of nicks between the hybridisation sites of C8–C9 primers was hence half of that for I2–I3 primers. Indeed, all the three samples did not amplify a product with I2–I3 primers, but they did with C8–C9 primers (Fig. 3, lanes 1–3). The SVBV nature of this product was confirmed by cleavage at unique *Taq*I site at nt 6961 (results not shown).

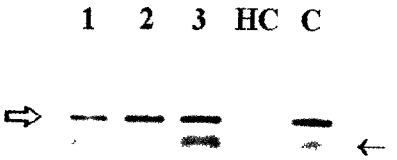

Fig. 3
Detection of SVBV in the samples from Slovakia by PCR and agarose gel electrophoresis
Negative picture. The samples (lanes 1–3), healthy control (lane HC), pSBVV-E3 positive control (lane C). Thick arrow marks the position of the 355 bp PCR product. Thin arrow marks the position of the primers

There are earlier reports on the occurrence of SVBV in European countries (Miller and Frazier, 1970). However, as they all were based on symptom observation only, their revision will be necessary. We confirmed the presence of SVBV in three European countries (Germany, Norway and the Czech Republic) previously (Mráz *et al.*, 1996b; Fig. 4).

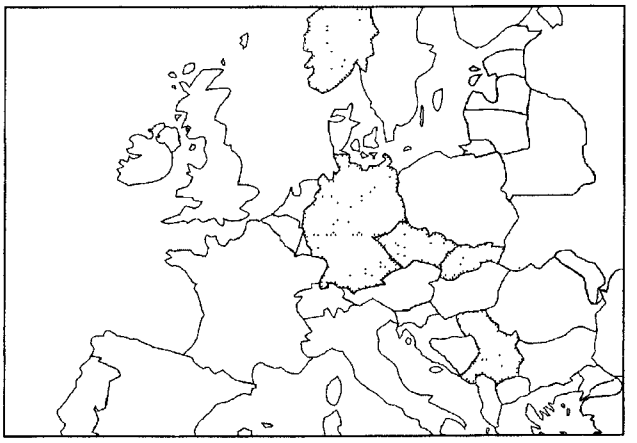

Fig. 4
Map of Europe with countries with the occurence of SVBV at the end od 1997
The occurence of SVBV confirmed by PCR and dot-blot hybridisation. SVBV-positive countries depicted as hatched areas.

As all these isolates originated from cultivated strawberries, it is highly probably that SVBV had spread through a propagation material. However, the source of the spread can be hardly traced. There is a suspicion of introduction of the German isolate by imported stock plants (D.G. Blystad, personal communication). The Norwegian isolate could come from Germany through Denmark, but there is lack of information about the occurrence of SVBV in Denmark. The Czech isolates could be introduced probably by stock plants imported from the western Europe, and the exchange of stock material between the Czech Republic and Slovakia could be the source of SVBV in the latter country. Identical sequences of the German, Norwegian and one of the Czech isolates with that of the isolate from the USA led us to speculate about the American origin of this virus in Europe (Mráz *et al.*, 1998).

The Serbian isolate detected in this study originated from Kukavica Mountain. There are no strawberry producing fields in the nearest vicinity of Kukavica Mountain which could serve as source of virus for vector transmission. According to Frazier and Morris (1987), SVBV is common in beach strawberry *F. chiloensis* (L.) along the Pacific Coast and it is presumed to infect wild strawberries in the western USA. Further investigation of its spread on wild strawberry is necessary.

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