DIAGNOSIS OF STRAWBERRY VEIN BANDING VIRUS BY A NON-RADIOACTIVE PROBE

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Summary. – A non-radioactive digoxigenin-labelled cDNA probe was prepared from genomic DNA of American isolate No. 45058 of strawberry vein banding virus (SVBV). Five different air-dried SVBV-containing strawberry leaf samples originating from National Clonal Germplasm Repository, Corvallis, USA, reacted positively in dot blot hybridization with this probe. Six of twelve strawberry samples from the Czech Republic exhibiting symptoms of SVBV – like infection gave positive reaction with this probe. Our results confirm the spread of SVBV in Central Europe and introduce the first reliable screening method for this virus.

Key words: strawberry vein banding virus; non-radioactive probe; hybridization; virus detection

Introduction

SVBV infection, firstly described by Frazier (1955), has been later observed on cultivated strawberries in North America, Australia, Brazil, Japan (Frazier and Morris, 1978) and recently in Europe (Converse, 1992; Honetšlegrová *et al.,* 1995). The virus may reduce both yield and fruit quality. Typical vein banding symptoms of the infection are shown in Fig. 1. The diagnosis of the virus should become routine, but classical detection methods so far used, such as grafting onto indicator clones, are time consuming. An antiserum against SVBV has not been prepared yet. SVBV usually occurs in host plants at low concentrations and in mixed infection with other viruses which tend to obscure SVBV symptoms (Frazier and Morris, 1987).

Here we present an application of a rapid and safe screening method, non-radioactive dot blot hybridization, to SVBV detection in plant samples from North America and Czech Republic.

Materials and Methods

Preparation of hybridization probe. A digoxigenin-labelled cDNA probe was prepared from genomic DNA of the American SVBV isolate No. 45058. Freeze-dried *Escherichia coli* JM83 transformed with pSVBV-E3 containing full-length SVBV DNA insert (Stenger *et al.,* 1988) was obtained from American Type Culture Collection. Originally, pSVBV-E3 plasmid was isolated using Wizard Minipreps (Promega) from *E. coli*. Genomic SVBV DNA was excised from this plasmid with *Eco*RI, cleaved with *Hind*III and recloned into pBS-.

The template SVBV DNA was obtained from pBS- by excision with *Hind*III, electrophoresis in low melting agarose and isolation. The probe was then synthesized using template SVBV DNA, the Klenow fragment of DNA

Abbreviations: BSA = bovine serum albumin; PBS = phosphate buffered saline; PVP = polyvinylpyrrolidone; SVBV = strawberry vein banding virus

Fig. 1
Symptoms of vein banding on *Fragaria vesca* EMK indicator clone after leaf grafting with Czech SVBV isolate F5

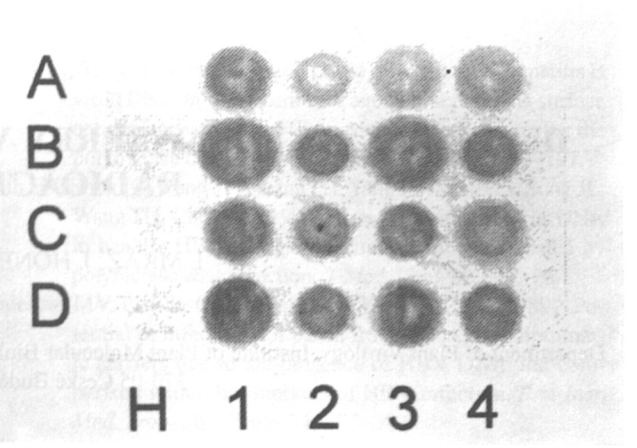

Fig. 3
Comparison of Czech SVBV isolates in dot blot hybridization
Dot rows H, 1-4: healthy control, *F. vesca* UC4 clone (H), isolates I8 (1), A89K (2), A52 (3) and American isolate 9044.001 (4). Dot rows A-D: identical samples.

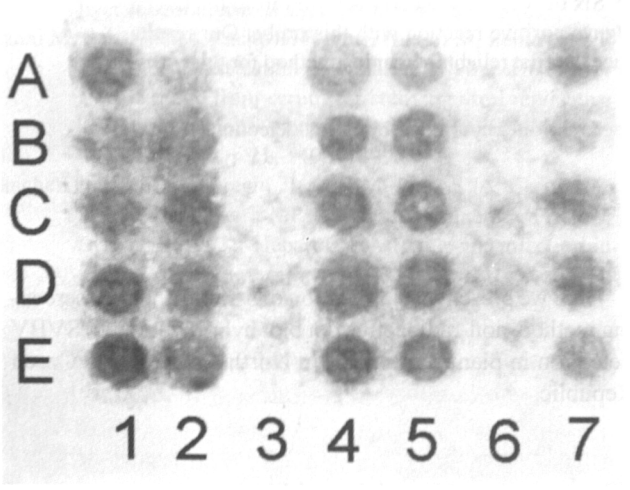

Fig. 2
Comparison of American SVBV isolates in dot blot hybridization
Dot rows 1-7: American isolate 9009.001 (1), American isolate 9044.001 (2), Healthy control, *F. vesca* UC4 clone (3,6), American isolate 9010.001 (4), American isolate 9016.001 (5), American isolate 9043.001 (7). Dot rows A-E: identical samples. Concentration of 38 µg of air-dried tissue per dot was employed.

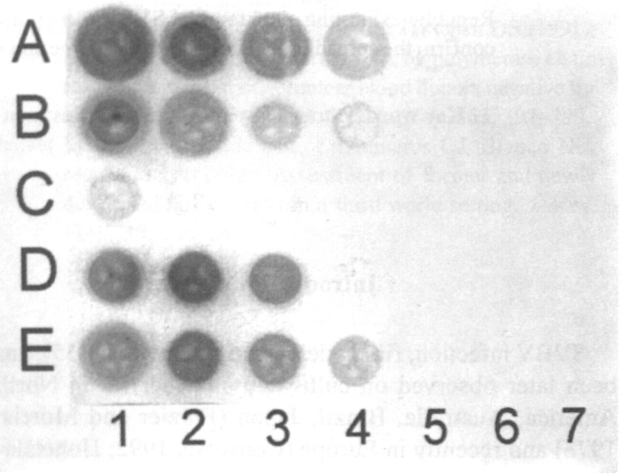

Fig. 4
Effect of dose of the sample on the detection of SVBV in dot blot hybridization
Dot rows 1-6: 250 µg (1), 200 µg (2), 150 µg (3), 100 µg (4), 50 µg (5) and 10 µg (6). Dot rows A-E: Czech isolate A52 (A), Czech isolate A89K (B), healthy control, *F. vesca* UC4 clone (C), American isolate 9044.001 (D,E).

polymerase and digoxigenin-labelled dUTP according to the standard protocol from Boehringer Mannheim Biochemica.

Leaf samples preparation. Leaf tissue (0.1 g) was homogenized in 1 ml of buffer (phosphate buffered saline (PBS), 0.05% (v/v) Tween 20, 1% (w/v) polyvinyl pyrrolidone (PVP), 1% (w/v) bovine serum albumin (BSA), 1 mol/l urea and 2.5% (v/v) Triton X-100 pH 7.4). Thirty-eight µg of an air-dried sample (all Americam samples and healthy control UC4) and 150 µg of a fresh sample (twelve Czech isolates and healthy control *Fragaria vesca* UC4) homogenized in buffer solution per dot were used. The employed buffer is recommended for degradation of caulimovirus inclusion bodies by Hull *et al.* (1976).

Dot blot hybridization. DIG Nucleic Acid Detection Kit and DIG DNA Labelling Kit were used in hybridization performed on positively charged nylon membranes (Boehringer Mannheim Biochemica) using the standard protocol from the manufacturer. Biospot Blotter DB-1000 (Bios, USA) was used in dot blot hybridization.

SVBV leaf samples. Air-dried American strawberry SVBV isolates 9009.001 (Vein banding – Western), 9010.001 (Vein banding – Western), 9016.001 (Vein banding – Alpine), 9043.001 (Vein banding, A-2/UC-1) and 9044.001 (Vein banding F-1/UC-6) were used. These samples originated from the National Clonal Germplasm Repository (N.C.G.R.), Corvallis, Oregon, USA, from the collection of strawberry viruses originally assembled by R.H. Converse. Twelve Czech virus isolates (A52, C3, D27, E21, F5, G4, I8 – cv. Redgauntlet, A59 – cv. Korona, A84, A46 – cv. Lidka, A89K – cv. Rujana and T22 – cv. Bounty) from strawberry plants which revealed SVBV – like symptoms after grafting onto indicator clones of *Fragaria vesca* were also employed. All these American and Czech isolates were tested in dot blot hybridization. *Fragaria vesca* UC4 cultivated from tissue culture acquired from N.C.G.R. was used as healthy control.

Results and Discussion

All five air-dried American SVBV isolates reacted positively in dot blot hybridization (Fig. 2). However, only six (A52, D27, G4, I8 – cv. Redgauntlet, A84 – cv. Lidka, A89K – cv. Rujana) of twelve Czech strawberry isolates exhibiting SVBV – like infection symptoms were positive in dot blot hybridization. The other six isolates did not react although they revealed marked symptoms of vein banding. The results of these studies seem to indicate that in these negative cases the SVBV - like symptoms could be caused by another virus or by a different strain of SVBV which did not react with the probe prepared against the American SVBV isolate. Indeed, in these negative Czech samples isometric virus particles of about 35 nm in diameter were observed in addition to 41 nm caulimovirus particles (Honetšlegrová *et al.*, 1995).

A comparison of dot blot hybridization of the American isolate 9044.001, Czech isolates I8, A52, A89K, and the healthy control (*Fragaria vesca* UC4) is shown in Fig. 3.

The sensitivity of the dot blot assay using different fresh samples in doses from 250 μg to 10 μg per dot is demonstrated in Fig. 4. The best performance in dot blot hybridization was obtained for 150 μg of fresh sample per dot. The minimum quantity required for reliable assay was 100 μg of fresh sample per dot.

Results of tests performed on American and Czech isolates prove the applicability of the dot blot hybridization using a non-radioactive probe for reliable detection of SVBV in leaf extracts. Positive reactions of 50% of samples originating from Czech Republic (A52, A89K, A84, D27, G4, I8) confirm the presence of SVBV in this country.

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