LETTER TO THE EDITOR

DETECTION OF STRAWBERRY VEIN BANDING VIRUS BY POLYMERASE CHAIN REACTION AND DOT BLOT HYBRIDIZATION

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Strawberry vein banding virus (SVBV) is one of seventeen members of the family *Caulimoviridae*. Natural infection with the virus is known in *Fragaria* species only. Infections caused by SVBV are often symptomless *(1)*, but their significance increases in mixed infections with strawberry crinkle or strawberry latent C viruses *(2,3)*. This virus has been originally found on strawberries in USA and firstly described by Frazier *(4)*, but it is probably world-wide distributed by planting or breeding materials. SVBV has been observed on cultivated strawberries in North America, Australia, Brazil, Japan *(5)* and recently in Europe *(6,7)*. The concentration of SVBV in infected plants is usually very low. Its detection by ELISA is impossible because of lack of specific antibodies.

Evidence of the caulimovirus nature of SVBV has been confirmed by its circular dsDNA genome, shape and size of viral particles *(8)*, presence of cytoplasmic inclusion bodies typical for caulimoviruses, and distant serological relationship with cauliflower mosaic virus (CaMV, *9*).

In this paper we present detection of SVBV by combination of two detection methods – polymerase chain reaction (PCR) and dot blot hybridization with a non-radioactive probe.

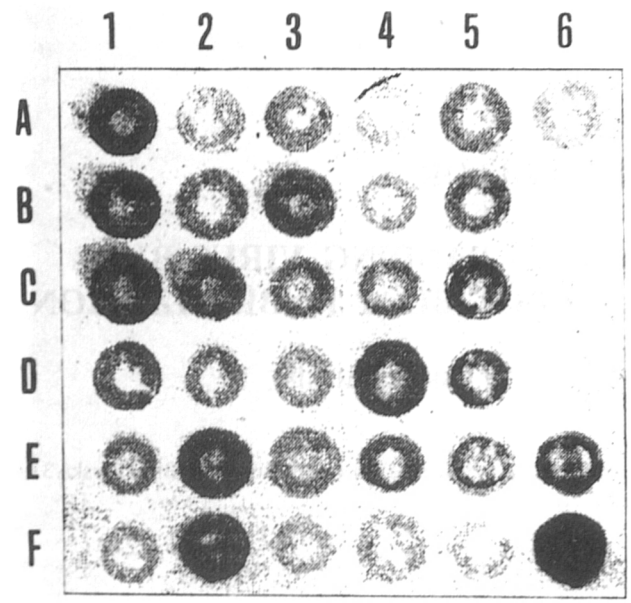
Key words: strawberry vein banding virus; non-radioactive hybridization probe; amplification; polymerase chain reaction; virus detection

Full length clone of the American SVBV isolate ATCC 45058 in pUC8 plasmid designed pSVBV-E3 *(10)* was obtained from the American Type Culture Collection (Corvallis, MD, USA). The German SVBV isolate from *F. ananassa* cv. Chandler (kindly provided by Dr. Jelkmann and Mr. Petruschke) is a field sample from Weinsberg near Stuttgart probably imported from another country. *F. vesca* indicator clones with vein banding symptoms after grafting with Czech (N1, N2, A52 and A89) and German (Chandler) SVBV isolates were used as positive controls. *F. vesca* UC4 clones derived from tissue culture were used as healthy controls. About 260 Czech plant samples suspected for SVBV infection originated from different localities of the Czech Republic were used in PCR and dot blot.

Six probes for all six putative SVBV genes were prepared from the pSVBV-E3 clone. Probes for ORFs I-III were generated by PCR amplification, purified from low-melting agarose and precipitated by ethanol. Probes for ORFs IV-VI were prepared as the *Hind*III fragments (nt 2407-3484, 3484-4312, and 6451-6810) of pSVBV-E3. All six fragments of SVBV genomic DNA were labelled using the Klenow enzyme and DIG-labelling kit (Boehringer Mannheim) with dUTP11-DIG according to the manufacturer's protocol *(11)*.

Total DNA was isolated from 100 mg of fresh frozen strawberry leaves using the alkaline lysis method *(12)*. Field samples were prepared in 50 µl of the amplification mixture con-

Abbreviations: CaMV = cauliflower mosaic virus; PCR = polymerase chain reaction; SVBV = strawberry vein banding virus



sisting of 1 x reaction buffer (10 mmol/l Tris-HCl pH 8.3, 50 mmol/l KCl, 2.5 mmol/l $MgCl_2$), 2.5 U/100 µl of *Taq* polymerase (Promega), 0.2 mmol/l each of dNTPs, 20 nmoles each of I2 and I3 primers (upstream I2 primer: 5'-CTGTCGACGAATGGGACAATGAAATGAG-3', ORF IV; downstream I3 primer: 5'-CAGAGCTCGTGAGGAGAACTTAGGACAC-3', ORF IV) and 1 µl of SVBV DNA from a strawberry sample. The amplified region was located between nt 2274 and nt 2876. The numbering of the amplified region is identical with that of the complete SVBV sequence of the American isolate ATCC 45058 *(13)*. The PCR was run in GeneAmp PCR System 9600 Amplifier (Perkin Elmer) for 35 cycles. Each cycle consisted of 94°C for 30 secs, 53°C for 60 secs and 72°C for 120 secs. Five µl of the isolated SVBV DNA or of the amplified product was used per dot in the dot blot hybridization.

The hybridization was performed on positively charged nylon membranes (Boehringer Mannheim) in a Biospot Blotter DB-1000 (BIOS Corporation, USA) using the manufacturer's protocol. About 260 PCR amplified Czech field samples were tested on the agarose gel and then by dot blot hybridization with the probes prepared from parts of genes I-VI of the American SVBV isolate ATCC 45058. Amplified as well as non-amplified isolates were used as positive controls in the assay.

Evaluation of some of the PCR products was ambiguous due to smeared bands on the agarose gel.

The figure demonstrates the dot blot hybridization of 26 positive amplified Czech SVBV isolates (1A-1F, 2A-2F, 3A-3F, 4A-4F, 5A and 5B), of 5 positive SVBV controls (5C-5F, 6A, 6E and 6F) and 3 negative SVBV controls (6B-6D) with the probe prepared from a part of gene IV. The same 26 samples of positive amplified Czech isolates yielded negative results with probes prepared from parts of genes I-III and V, VI (results not shown).

All 6 probes reacted positively with amplified as well as with non-amplified positive control SVBV isolates. We assume that the concentration of SVBV in the positive controls was extremely high. When 260 amplified Czech field samples suspected for SVBV infection were subjected to the dot blot hybridization, we obtained positive signals only with a corresponding probe prepared from DNA isolated from part of gene IV. All amplified plant samples tested by the dot blot hybridization gave unambiguously positive or negative signals. There were no differences between the results obtained by PCR and dot blot assay, but some ambiguous results obtained by PCR were cleared by the dot blot assay.

Our results show that for a routine dot blot test of field samples, it is advantageous to use an amplified virus nucleic acid and a homologous probe. Without amplification we can use a nucleic acid extract or crude sap *(11)* from grafted indicator clones but grafting is more laborious and time consuming.

The sensitivity of DNA hybridization is greatly enhanced by PCR, which is important for low virus concentration in field samples, where PCR amplification is necessary to obtain a positive signal. The probe selectivity and correct PCR amplification are confirmed by the fact that the probe prepared from a particular gene reacted only with those samples, where the same gene was amplified. We may conclude that the combination of PCR and hybridization techniques provides a reliable method for SVBV detection.

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