Variability in Coat Protein Sequence Homology Among American and European Sources of Strawberry Vein Banding Virus

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ABSTRACT

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Strawberry vein banding virus (SVBV) isolates from North America, Czech Republic, Norway, and Germany were collected and their variability was determined by dot blot hybridization and confirmed by sequencing of a 431-nucleotide fragment from the middle part of the coat protein gene. Two different substitutions were found between the American and two Czech SVBV isolates, but the other isolates were identical in the compared region to the American isolate. Digoxigenin-labeled probes were prepared from these isolates and used for hybridization with polymerase chain reaction-amplified fragments of 26 Czech SVBV field isolates. No significant differences in the hybridization signal were found with any combination of samples and probes. These results show that the European isolates probably originate from a common ancestor and may have been introduced to Europe from America with planting or breeding material.

Strawberry vein banding virus (SVBV) is one of 17 definitive or putative members of the family *Caulimoviridae*. Strawberry (*Fragaria* sp.) is the only known natural host and the strawberry aphid (*Chaetosiphon* sp.) is the only known natural vector in the United States (5). SVBV has been observed on cultivated strawberries in North America, Australia, Brazil, Japan (6), and, recently, in Europe by Blystad and Schia (4) and by Honetšlegrová et al. (9). There are no commercially available antibodies for serological detection of SVBV. Its incidence has been determined by grafting to indicator plants. Despite its unreliability, grafting onto indicator clones has been the only available detection method for SVBV. Knowledge of the complete nucleotide sequence of SVBV (16) allows the development of less laborious and more sensitive hybridization and polymerase chain reaction (PCR)-based detection methods (13,17). Based on samples tested from five European countries, the occurrence of SVBV in planting stocks is probably much higher than previously reported. Sequence comparison of 1 American and 5 European (Czech, Ger-

man, and Norwegian) SVBV isolates and hybridization screening of field samples presented in this paper are the first attempts to determine the variability of SVBV at the molecular level.

MATERIALS AND METHODS

SVBV isolates. A full-length clone of the American SVBV isolate in pUC plasmid designated pSVBV-E3 (19) was obtained from the American Type Culture Collection (ATCC; product No. 45058 Rockville, MD). The German isolate of SVBV (provided by Jelkmann and Petruschke) was a field sample from cv. Chandler from Weinsberg near Stuttgart. The Norwegian isolate of SVBV came from cv. Mimek and originated from southern Norway (provided by D. R. Blystad).

Field samples. Twenty-six field samples giving positive results both in PCR and dot blot hybridization with a probe prepared previously (13) originated from localities in East and South Bohemia. Additional positive controls were taken from *F. vesca* indicator clones, where vein banding symptoms occurred after grafting with the Czech (N1-*F. vesca* FV 72, A52-*F. vesca* UC4, A89-*F. vesca* *semperflorens* cv. Rujana), German (*F. ananassa* Duch. cv. Chandler), and Norwegian (*F. ananassa* Duch. cv. Mimek) SVBV isolates. *F. vesca* plants, derived from a tissue culture, were used as healthy controls.

Primers and PCR. Primers I2 and I3 were designed from the SVBV coat protein gene (ORF IV on the complete sequence): I2-5'-²²⁷⁴GAATGGGACAATGAAATGAG 3', I3-5'-²⁸⁴⁷GTGAGGAGAAGCTTAGGAC-

AAC 3'. Numbering is identical with the complete SVBV sequence (16). Fifty µl reaction mixture in 1× reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂) containing 2.5 U/100 µl *Taq* polymerase (Promega Corp., Madison, WI), 0.2 mM each of dNTPs, and 20 nmol upstream and downstream primers, were mixed with 1 µl total DNA isolated from 100 mg frozen SVBV-infected strawberry leaves. The PCR was performed in a GeneAmp PCR system 9600 thermal cycler (Perkin Elmer, Norwalk, CT) for 35 cycles of 94°C for 30 sec, 53°C for 60 sec, and 72°C for 120 sec.

Preparation of hybridization probes and samples. The hybridization probes of the coat protein gene were prepared by labeling agarose gel-purified 573 base pair (bp) PCR products of the ATCC 45058, A52, Chandler, and Mimek SVBV isolates using Klenow enzyme and the digoxigenin (DIG) DNA Labeling Kit (Boehringer, Mannheim, Germany) with dUTP¹¹-DIG according to the manufacturer protocol (13). Total DNA was isolated from 100 mg fresh or frozen leaves using the alkaline lysis method (20). Five µl isolated SVBV DNA or 5 µl I2-I3 amplified product were used per dot in dot blot hybridization.

Dot blots and sequencing. Hybridization was performed on positively charged nylon membranes (Boehringer) and a Biospot blotter DB-1000 (BIOS Corporation, New Haven, CT) was used in dot blot using the standard protocol from Boehringer. The PCR products of the ATCC 45058, A52, A89, N1, Chandler, and Mimek isolates, were sequenced directly using a Sequenase DNA sequencing kit (Version 2.0, United States Biochemical Corp., Cleveland) and by both I2 and I3 amplification primers.

RESULTS AND DISCUSSION

We collected and tested about 1,300 samples of cultivated strawberry plants and did not observe symptoms specific for SVBV infection in all samples. Most plants in which SVBV was detected were symptomless (9).

Amplified DNA from 26 Czech SVBV samples were hybridized with probes prepared from the American (Fig. 1), Czech, German, and Norwegian isolates. All samples gave a signal similar to positive controls with each of the probes. Differences in the signal strength were due to different

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concentrations of the virus among the samples, rather than to different reactivity with the probes. These results indicated that all isolates are highly conserved.

Amplification of the SVBV nucleic acid from field samples was necessary before hybridization because virus concentrations were low (14). On the other hand, infected indicator clones of *F. vesca*, used as positive controls, showed vein banding symptoms and gave positive signals in hybridization assays without previous amplification. Hybridization and PCR assays were both negative in samples of healthy controls.

SVBV appears to be native to North America, where it occurs in beach strawberries, *F. chiloensis* (L.) (12). Several strains of SVBV were described originally, but only two of them differ in symptoms on indicator plants: isolates from California caused characteristic vein banding and are known as western types, while isolates from the east coast of the United States cause leaf curling and are known as eastern types (6). The ATCC 45085 isolate, whose entire genome was recently sequenced (16), was shown to be a western type. This isolate hybridized well with the European isolates.

We compared a 431-nucleotide-long sequence of the middle part (nucleotide position 2347-2778) of the 1521 nucleotide SVBV coat protein gene (Fig. 2). Four isolates were identical in this region: N1 (Czech), Chandler (German), Mimek (Norwegian), and the ATCC 45058 (American) isolate. Two different substitutions were found on the other Czech isolates: TCT→TTT at position 2425 of isolate A89, which would change the amino acid Ser₁₇₉→Phe, and AAA→ATA at position 2647 of isolate A52, leading to a Lys₂₅₃→Ile change. These two nucleotide substitutions represent only a 0.46% variability on the 431 bp sequence. Based on this data, we assume that all isolates including the Czech A89 and A52 belong to the same western type. Currently, only the western type of SVBV is maintained at ATCC (J. Postman, *personal communication*), and therefore the eastern SVBV type was not included in these tests.

The observed variability of SVBV seems to be very low for such geographically distant isolates. Therefore, the variability within isolates of another member of the family *Caulimoviridae*, cauliflower mosaic virus (CaMV), which is also worldwide in distribution, was tested. Many isolates of CaMV differ in symptom expression (1), host range (18), or vector transmission (10). Nucleotide sequences of the two closely related G1 (8) and BBC (3) isolates differ at 13 positions in the region corresponding to the 2347-2778 region of the SVBV. This is a higher variability (2.7%) than was observed among the SVBV isolates. Analysis of the complete nucleotide sequence of SVBV indicated

that the genome arrangement and function of putative proteins should be similar to CaMV (16). If the SVBV population in America and Europe existed independently over a long period of time, then a variability similar to CaMV would be predicted. Occurrence of identical sequences cannot be explained by identical evolution alone. More likely SVBV was introduced to Europe from North America. Frazier and Morris (6) stated that SVBV was probably introduced to Australia, Brazil, and Japan by planting or breeding material. The German Chandler SVBV isolate also origi-

nated from a third country (Jelkmann, *personal communication*). The SVBV in Norway occurred in fields where plants were reimported from Denmark (D. R. Blystad, *personal communication*). A similar situation occurred in nurseries in the Czech Republic, where some new cultivars had been imported from other countries.

There were no diagnostic symptoms on commercial strawberries. But typical vein banding symptoms are produced best on indicators *F. vesca* or *F. virginiana* clones. However, symptoms vary in severity de-

Fig. 1. Dot blot hybridization of 26 field samples (vertically left to right) (1A to 5B) with probe prepared from the American Type Culture Collection 45058 strawberry vein banding virus (SVBV) isolate; (5C to 6A) positive controls N1, A89, A52, Chandler, and Mimek; (6B to 6D) healthy control; (6E) control DNA-labeled; and (6F) pSVBV-E3.

Fig. 2. Localization of the compared fragment of coat protein gene (CP) within the open reading frames (ORF) III and V of the strawberry vein banding virus (SVBV) genome. Comparison of nucleotide sequence of the American Type Culture Collection 45058 isolate (45058); German isolate Chandler (Chan); Norwegian isolate Mimek; and Czech N1, A52, and A89 isolates. Position where substitutions were observed and supposed amino acid changes are marked. Amplification primers I2 and I3 are marked as arrows. Nucleotides are counted according to complete nucleotide sequence of SVBV.

pending on the isolate of the virus. In the past, the economic importance of SVBV was thought to be minor because of the low incidence in commercial strawberries. When alone, it reduces runner production and vigor of plants (11), but the significance of SVBV increases in mixed infections with strawberry crinkle, strawberry latent C virus, and other strawberry viruses by reducing both yield and fruit quality (2,7).

Propagules of strawberry plants are routinely screened for the presence of nepoviruses such as arabis mosaic, raspberry ringspot, strawberry latent ringspot, and tomato black ring. However, asymptomatic infection by western type SVBV or other viruses which do not exhibit visible symptoms escape elimination during propagation, and the viruses are further spread with planting material. Furthermore, propagation stock plants are a long-term source of these viruses. SVBV has been listed as a quarantine pest by the European and Mediterranean Plant Protection Organization since 1978 (15). We recommend that at least one nucleic acid-based test (either PCR or hybridization) for SVBV be used to complement graft indexing of stock plants.

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