LETTER-TO-THE-EDITOR

STRAWBERRY VEIN BANDING VIRUS DETECTION BY DOUBLE ANTIBODY SANDWICH ELISA WITH ANTIBODIES TO RECOMBINANT COAT PROTEIN

K. PETRZIK*, D. KUBELKOVÁ, I. MRÁZ

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

Received January 30, 2002; accepted May 30, 2002

Key words: strawberry vein banding virus; recombinant coat protein; Pichia pastoris; Escherichia coli; DAS-ELISA

Besides strawberry quarantine viruses the strawberry vein banding virus (SVBV, species *Strawberry vein banding virus*, genus *Caulimovirus*, family *Caulimoviridae*) is the only one for which a specific serological assay is not available. The previously reported cross-reactivity of SVBV with a cauliflower mosaic virus (CaMV) antiserum has been observed in a few field samples which gave an A_{405} value over 1.0 in ELISA (*1, 2*). However, this result did not correlate with those of grafting tests; hence a CaMV antiserum has not been recommend for SVBV diagnosis. There are no data on the virus distribution and concentration in the infected plant as well as on the evaluation of the best timing for sample collection. Our previous results have shown sporadic occurrence of caulimovirus-like isometric virions in purified preparations of SVBV (*3*).

The main goal of this work was the development of a serological test for SVBV. To prepare a recombinant SVBV coat protein (CP) we constructed an expression cassette in the pPICZαA vector (Invitrogen) which allowed a secreted production of CP in the *Pichia pastoris* yeast and a metal affinity purification of histidine-tagged CP. SVBV CP was secreted in amounts of 1 to 2 mg per liter.

To prepare a SVBV CP antibody a rabbit was immunized with three 0.4 mg doses of a dialyzed purified CP in one-week intervals and the antibody was precipitated from the serum with ammonium sulphate (*4*).

To confirm the anti-SVBV CP nature of this antibody a recombinant SVBV CP expressed in *Escherichia coli* using the pET16b vector (Novagen) with an expression cassette containing the full-length SVBV CP gene without any tagged peptides and a SVBV-infected *Fragaria vesca* plant were used. Series of CP-enriched centrifugal fractions prepared from an *E. coli* culture as described previously (*5*) and a leaf homogenate from *F. vesca* clone 9010 obtained from the National Clonal Germplasm Repository, Corvallis, OR, USA (NCGR) diluted 1:1, 1:10, 1:100, 1:200, and 1:400 in a phosphate buffer (the figure, row L) were spotted on a membrane and detected with the SVBV CP antibody (primary antibody) and an alkaline phosphatase-conjugated goat anti-rabbit antibody (secondary antibody, Sigma-Aldrich Chemie), both diluted 1:3,000. The SVBV CP antibody reacted strongly with the centrifugal fractions sedimenting at 10 and 2 x 10^4 x g, respectively, (the figure, rows 98 and 99), similarly as in (*5*). A SVBV-positive signal was visible also with a leaf homogenate from the infected but not from the healthy *F. vesca* plant up to the 1:200 dilution

*E-mail: petrzik@umbr.cas.cz; fax: +420385-300363.

Abbreviations: CaMV = cauliflower mosaic virus; CP = coat protein; DAS-ELISA = double-antibody sandwich ELISA; SVBV = strawberry vein banding virus

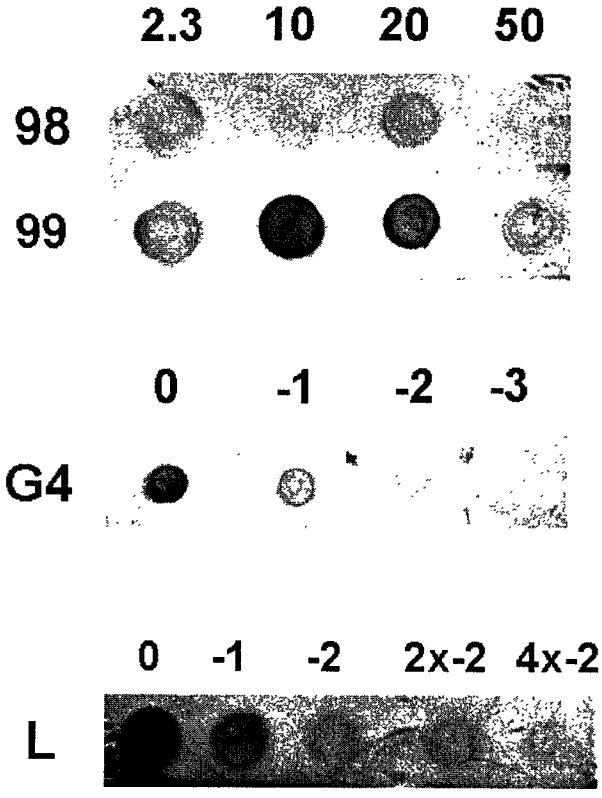

Fig. 1

(the figure, row L) and, naturally, with the proteins secreted by *P. pastoris* clone G4 (the figure, row G4).

The dilutions 1:10, 1:20, and 1:50 of fresh tissues from SVBV-infected *F. vesca* clones 9010, 9016, 9043, and 9044, obtained from NCGR, two naturally infected strawberry cultivars and a CaMV sample were subjected to a double-antibody sandwich ELISA (DAS-ELISA) (*6*). Both the prepared rabbit SVBV CP antibody (the coating, primary antibody) and the alkaline phosphatase-labeled SVBV CP antibody (the secondary antibody) were diluted 1:5,000 in the BIOREBA buffer. The healthy plants as well as CaMV, related to SVBV, showed an A_{405} value of about 0.14± 0.1, while the diseased plants gave significantly higher values, from 0.8 to more than 2.0, depending on the plant (data not shown). The antiserum was SVBV-specific and therefore suitable for the detection of SVBV in the ELISA described here.

Acknowledgement. This work was supported by the grant No. 522/99/0437 of the Grant Agency of the Czech Republic.

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