Double-Stranded RNA in Strawberry Plants Infected with Strawberry Mild Yellow-Edge Virus

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ABSTRACT

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Double-stranded RNA (dsRNA) has been isolated from strawberry plants infected with strawberry mild yellow-edge virus but not from virus-tested plants. Three major dsRNA bands were present in 20 independent extractions from two cultivars. These bands had relative molecular masses of 3.8, 2.8, and 1.3×10^6 Da. These dsRNA species are similar to those previously reported for dsRNA from plants infected with the well-characterized luteoviruses barley yellow dwarf virus and beet western yellows virus.

Strawberry mild yellow-edge virus (SMYEV) is one of the most common viruses in cultivated strawberries (*Fragaria* × *ananassa* Duch.). Most strawberry cultivars infected with SMYEV are symptomless, but complexes of SMYEV with other viruses have been reported to reduce plant vigor, yield, and fruit quality (12). SMYEV is transmitted in a persistent manner by the strawberry aphid, *Chaetosiphon fragaefolii* (Cockrell), and two other aphid species but not by sap inoculation (12). Virus particles found in the infected tissue were isometric and limited to the phloem cells (19; E. R. Florance, T. C. Allen, and R. H. Converse, *unpublished*). Virus particles isolated from infected plants are serologically related to beet western yellows virus (BWYV) (16). Because of the difficulty in purifying phloem-limited viruses occurring at very low concentrations in the infected tissue, only partial purification of one isolate of SMYEV has been reported (10). However, no information is currently available on its physical composition. Based on particle morphology, serology, and symptomatology, SMYEV has been considered a possible member of the luteovirus group (11,16).

Detection of SMYEV in infected cultivars is based on transmission of the virus to sensitive clones of *F. vesca* L., either by leaflet grafting or by aphid vectors. In this paper, the isolation of double-stranded RNA (dsRNA) from symptomless, infected strawberry plants is reported. The pattern of the isolated dsRNAs further supports the generally held hypothesis that SMYEV should be included in the luteovirus group.

MATERIALS AND METHODS

Two isolates of SMYEV were used in this study, one detected in a commercial strawberry field of the cultivar Aliso at Tira, Israel, and the other in strawberry plants of the cultivar Chandler imported from California. Infected plants were maintained in an insect-proof screenhouse. Leaflets from infected plants were grafted onto *F. vesca* clone UC-4 and onto *Duchesnea indica* (Andr.) Focke to ensure that the SMYEV isolates were free of other known viruses. SMYEV induces symptoms on UC-4 (5) and is usually symptomless on *D. indica* (14). For comparison, virus-tested plants were tested and found free of SMYEV, strawberry mottle virus (SMV) (17), and tobacco streak virus (TSV) (15). These plants were obtained using thermotherapy followed by shoot-tip culture. Both infected and virus-tested plants were propagated vegetatively in an insect-proof screenhouse.

The method used to extract dsRNA was modified from that of Morris and Dodds (13). Leaves were frozen and kept at −20 C or preferably lyophilized and pulverized with a Thomas-Wiley laboratory mill (intermediate model) (General Electric Corp.). The resulting powder was stored in a desiccator at 4 C. For extraction of dsRNAs, samples (50 g fresh weight or about 15 g of dry powder) were suspended in an extraction medium consisting of 200 ml of 2XSTE (STE:50 mM Tris-Cl, 100 mM NaCl, and 1 mM NaEDTA, pH 7.0), 0.1% Cellulase R-10 (Yakult Biochemicals, Nishinomiya, Japan), 0.1% diethyl dithiocarbamic acid and 0.1% mercaptoethanol and were incubated for 3 hr at 25 C. Then, 150 ml of STE-saturated phenol, pH 7.0, containing 0.1% 8-hydroxyquinoline and 130 ml of chloroform were added and the mixture was stirred for 1 hr at 4 C, centrifuged at 8,000 *g* for 15 min, and the aqueous phase collected. Ethanol and sodium acetate were then added while stirring to a final concentration of 33% and 0.1 M, respectively, at pH 5.5. The mixture was stored overnight at −20 C before collecting the precipitate by centrifuging at 12,000 *g* for 30 min. The resulting gelatinous pellet was suspended in 50 ml of STE containing 15% (v/v) ethanol and stirred for 1 hr at 4 C. Cellulose powder (Whatman CF-11) was added to the 15% ethanol solution (0.5 g/10 g fresh weight of tissue) and stirred for 1 hr at 4 C. The cellulose with bound dsRNA was then collected by centrifugation at 3,000 *g* for 10 min, washed three times with 15% ethanol-STE, and the dsRNA was eluted with 8 ml of STE buffer. The dsRNA was precipitated with 2.5 volumes of ethanol, made to 0.1 M sodium acetate, pH 5.5, and kept at −20 C overnight. The precipitated dsRNA fraction was collected by centrifugation at 12,000 *g* for 30 min and treated with DNase (Sigma) at 5 μg/ml in STE containing 30 mM $MgCl_2$ at 37 C for 45 min. Ethanol was added to 15% and the mixture was passed again through a CF-11 column (1 g of dry powder), and the dsRNA eluted and precipitated with ethanol as described previously. The final pellet was dissolved in 50 μl of electrophoresis buffer containing 20% glycerol. Nucleic acids were electrophoresed in 5% polyacrylamide gels (18 × 16 × 1.5 mm) using the buffer system of Loening (8). Electrophoresis was for 18 hr at 40V (constant voltage) at 4 C. Molecular weight markers were dsRNAs from *Nicotiana tabacum* L. 'Samsun' leaves infected with tobacco mosaic virus (TMV) and *Pachystachys coccinea* Nees leaves infected with cucumber mosaic virus (CMV) (1). After electrophoresis, the gels were stained with ethidium bromide (0.5 μg/ml) and the bands were visualized by ultraviolet fluorescence.

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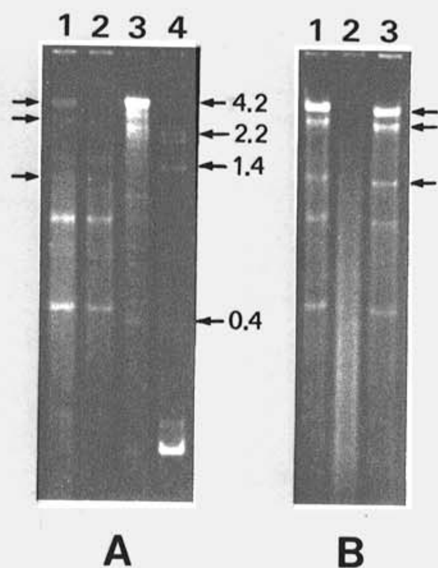

Fig. 1. Polyacrylamide gel electrophoresis (5%) of dsRNAs extracted from: **A,** SMYEV-infected Aliso strawberries (lane 1), virus-tested Aliso strawberries (lane 2), TMV-infected *Nicotiana tabacum* (4.2 and 0.4×10^6 Da) (lane 3), CMV-infected *Pachystachys coccinea* (2.2 and 1.4×10^6 Da) (lane 4). **B,** RNase A and DNase-treated dsRNAs extracted from SMYEV-infected Aliso strawberries. SMYEV-specific dsRNA treated with DNase (lane 1); SMYEV-specific dsRNA treated with RNase A in 15 mM NaCl, 1.5 mM trisodium citrate (lane 2); as for 2 but in 450 mM NaCl, 45mM trisodium citrate (lane 3). Samples were analyzed by electrophoresis in a slab gel for 18 hr at 50V at 4 C followed by staining in ethidium bromide (0.5 μg/ml). Arrows on left of A and right of B indicate the major SMYEV-specific dsRNA bands 3.8, 2.8, and 1.3×10^6 Da.

RESULTS AND DISCUSSION

Double-stranded RNA extracts from equal fresh weights of SMYEV-infected and virus-tested strawberry plants were compared by gel electrophoresis (Fig. 1A). The detectable amounts of dsRNAs were very low and could be visualized well only when the RNA obtained from 15–20 g (fresh weight) was loaded into one slot on the gel. Application of a similar extraction method to *N. tabacum* and *P. coccinea* infected with TMV and CMV, respectively, enabled clear detection of dsRNA bands even when dsRNA resulting from 0.1 g (fresh weight) was loaded into one slot of the gel.

Three major high-molecular-weight dsRNA bands were consistently found in each of 20 independent extractions made throughout the year from SMYEV-infected plants of cultivars Aliso and Chandler but not in 10 independent extractions of virus-tested Aliso plants. No differences were observed in the mobility of the dsRNA species of the two isolates studied (data not shown). In addition, two low-molecular-weight dsRNA bands were found in extracts from both SMYEV-infected and virus-tested plants. The three major dsRNA bands found in infected plants were 3.8, 2.8, and 1.3×10^6 Da, and the two dsRNA bands found in extracts both from infected and virus tested plants were 0.9 and 0.5×10^6 Da.

The double-stranded nature of these bands was verified by RNase A treatment under high- and low-salt conditions (Fig. 1B). The dsRNA purified from SMYEV-infected plants was degraded by RNase A (10 μg/ml) incubated in 15 mM NaCl, 1.5 mM trisodium citrate but was unaffected by RNase A incubated in 450 mM NaCl, 45 mM trisodium citrate.

The dsRNA pattern obtained from SMYEV-infected plants is similar to those previously reported for RNA from plants infected with the well-characterized luteoviruses barley yellow dwarf (BYDV) (6) and BWYV (4) (Table 1). A similar pattern was reported recently for dsRNA from banana bunchy top-diseased banana plants (2). The amount of dsRNA in SMYEV-infected tissue was very low and similar to that reported for other luteoviruses (2,4,6).

TABLE 1. Comparison of molecular weights of major dsRNA species extracted from strawberry plants infected with strawberry mild yellow-edge virus (SMYEV) and the luteoviruses barley yellow dwarf virus (BYDV) and beet western yellows virus (BWYV)

Band	SMYEV[a]	BYDV		BWYV[b]	
		Group 1[c]	Groupe 2[d]	STFL	ST9
1	3.80[e]	3.60	3.80	3.60	3.60
2	2.80	2.00	1.60	···	2.20
3	1.30	1.20	1.20	1.40	1.40
4	0.90	···	···	···	···
5	···	0.55	0.55	···	···
6	0.5	0.50	···	···	0.46

[a] Present study.
[b] Falk and Duffus (4).
[c] Gildow et al (6), strains MAV, PAV, and SEV.
[d] Gildow et al (6), strains RPV and RMV.
[e] Relative molecular mass.

The presence of dsRNAs in apparently virus-free plants was reported for various plant species (4,7,18). These reports reemphasize the need for controls when using the presence of dsRNAs in plants as a diagnostic indicator of virus infection (3). Several hypotheses regarding the origin of dsRNA in virus-free plants have been suggested (18). In the case of strawberry plants, the possibility that a latent or cryptic virus, which fails to induce symptoms in indicator clones, is present in some virus-tested cultivars (9) should also be considered.

The presence of a distinct banding pattern of SMYEV-dsRNA extracted from strawberry leaf tissue, which is notorious for its high content of phenolic compounds, will eventually allow rapid detection of the virus in infected symptomless plants and may enable characterization of the viral genome.

LITERATURE CITED

1. Bar-Joseph, M., Rosner, A., Moscovitz, M., and Hull, R. 1983. A simple procedure for the extraction of double-stranded RNA from virus-infected plants. J. Virol. Methods 6:1-8.
2. Dale, J. L., Phillips, D. A., and Parry, J. N. 1986. Double-stranded RNA in banana plants with bunchy top disease. J. Gen. Virol. 67:371-375.
3. Dodds, J. A., Morris, T. J., and Jordan, R. L. 1984. Plant viral double-stranded RNA. Annu. Rev. Phytopathol. 22:151-168.
4. Falk, B. W., and Duffus, J. E. 1984. Identification of small single- and double-stranded RNAs associated with severe symptoms in beet western yellows virus-infected *Capsella bursa pastoris*. Phytopathology 74:1224-1229.
5. Frazier, N. W. 1974. Six new strawberry indicator clones evaluated for the detection and diagnosis of twelve graft-transmissible diseases. Plant Dis. Rep. 58:28-31.
6. Gildow, F. E., Ballinger, M. E., and Rochow, W. F. 1983. Identification of double-stranded RNAs associated with barley yellow dwarf virus infection of oats. Phytopathology 73:1570-1572.
7. Ikegami, M., and Fraenkel-Conrat, H. 1979. Characterization of double-stranded ribonucleic acid in tobacco leaves. Proc. Natl. Acad. Sci. USA 76:3637-3640.
8. Loening, U. E. 1967. The fractionation of high-molecular weight ribonucleic acid by polyacrylamide gel electrophoresis. Biochem. J. 102:251-257.
9. Martin, R. R., and Converse, R. H. 1982. Evidence for an unknown latent virus in strawberry. (Abstr.) Phytopathology 72:989.
10. Martin, R. R., and Converse, R. H. 1985. Purification, properties and serology of strawberry mild yellow-edge virus. Phytopathol. Z. 114:21-30.
11. Matthews, R. E. F. 1982. Classification and nomenclature of viruses. Intervirology 17:1-200.
12. Mellor, F. C., and Frazier, N. W. 1970. Strawberry mild yellow-edge. Pages 14-16 in: Virus Diseases of Small Fruit and Grapevines. N. W. Frazier, ed. University of California, Division of Agricultural Sciences, Berkeley.
13. Morris, T. J., and Dodds, J. A. 1979. Isolation and analysis of double-

stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69:854-858.
14. Spiegel, S. 1986. An improved indexing procedure for some strawberry viruses in a hot climate area. Acta Hortic. 186:77-82.
15. Spiegel, S., and Cohen, J. 1985. Occurrence of tobacco streak virus in strawberries in Israel. Plant Dis. 69:448-449.
16. Spiegel, S., Cohen, J., and Converse, R. H. 1986. Detection of strawberry mild yellow-edge virus by serologically specific electron microscopy. Acta Hortic. 186:95.
17. Spiegel, S., and Frank, A. 1982. Virus and virus-like diseases in commercial strawberry cultivars in Israel. Acta Hortic. 129:99-101.
18. Wakarchuk, D. A., and Hamilton, R. I. 1985. Cellular double-stranded RNA in *Phaseolus vulgaris*. Plant Mol. Biol. 5:55-63.
19. Yoshikawa, N., Ohki, S. T., and Inouye, T. 1984. Luteovirus-like particles in phloem tissue of strawberry mild yellow-edge virus infected plants. Ann. Phytopathol. Soc. Jpn. 50:659-663.

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