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A survey of grapevine fanleaf nepovirus isolates for the presence of satellite RNA

by

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Summary: Grapevine fanleaf virus (GFLV) isolates from different geographical origins were surveyed for natural occurrence of satellite RNA. The results of molecular hybridization assays indicated that 5 isolates out of 34 tested, support the multiplication of a satellite RNA, both in *Chenopodium quinoa* and grapevine. The satellite molecules appear to have a high degree of sequence homology with, and the same size of, the satellite RNA supported by GFLV-F13 strain, isolated and characterized in France.

Key words: fanleaf, nepovirus, satRNA, hybridization, molecular probes, diagnosis.

Introduction

It is known that grapevine fanleaf nepovirus (GFLV), the agent of a severe disease of *Vitis* supports, similarly to many other nepoviruses (MAYO 1991), the multiplication of a satellite RNA (satRNA) (PINCK *et al.* 1988).

GFLV satRNA is a linear molecule 1114 nucleotide long which was been thoroughly characterized and sequenced (PINCK *et al.* 1988; FUCHS *et al.* 1989). Recent studies, in which biologically active transcripts were used, indicated that it may have a modulating effect on the pathogenicity of the helper virus (FUCHS *et al.* 1991 a).

The natural distribution of GFLV satRNA is largely unknown, although molecular probes have been developed which allow for its detection in naturally and artificially infected hosts (FUCHS *et al.* 1991 b). As reported in the present paper, 34 GFLV isolates of different geographical origin were analyzed for natural occurrence of satRNA by molecular hybridization with a specific probe.

Materials and methods

Virus sources: Naturally infected grapevines were from a collection of the University of Bari (Table) and were selected so as to include plants with a wide variety of symptomatologies, from very mild to very severe. All these vines were growing on their own roots, and had been propagated without further manipulation (e.g. grafting onto rootstocks) from canes collected in the country of origin. Healthy grapevines were from heat-treated sources checked for the absence of GFLV. Virus strains were recovered by mechanical inoculation and maintained in *Chenopodium quinoa* in a temperature-controlled glasshouse.

Virus purification: Purification of virus was from infected *C. quinoa* as described by PINCK *et al.* (1988): after clarification with butanol, polyethyleneglycol (PEG m.wt. 6000) precipitation and differential centrifugation, nucleoprotein compo-

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nents were separated in a 10—50 % linear sucrose gradient. Gradient tubes were fractionated with an ISCO 640 apparatus and virus containing fractions were concentrated by centrifugation at 302,000 *g* for 90 min.

RNA extraction: RNAs were extracted from purified virus particles with the phenol/SDS method, and ethanol precipitated (DIENER 1968).

RNAs were purified from *C. quinoa* and grapevine tissue essentially according to WHITE and KAPER (1989): after the phenol-chloroform extraction the aqueous phase was again extracted with chloroform, then ethanol precipitated. After centrifugation, the pellet was washed twice with 3 M sodium acetate pH 5.8, once with 70 % ethanol and resuspended in distilled H_2O.

Table

Presence of satellite RNA in GFLV isolates from different geographical origins

GFLV isolate	Origin	Symptoms	Detection of GFLV satRNA by molecular hybridization C. quinoa	Grapevine
Aq 4	Italy	Ds	+	+
Aq 7	Italy	Ds	+	+
Ba 6	Italy	Dm	-	-
Barile 1103 P.	Italy	Dm	-	-
Cannonau 1 A	Italy	VB	-	-
Martelli V. B.	Italy	VB	-	-
Mt 42	Italy	Di	+	+
Na 4	Italy	Di	-	-
Pa 22	Italy	Dm	-	-
Prosecco	Italy	VB	+	+
Sangiovese P.	Italy	Ds	-	-
Ss 1	Italy	YM	-	-
Sv 1	Italy	Ds	-	-
Ta 11	Italy	Dm	-	-
E 3	Spain	Dm	-	-
H 9	Hungary	Di	-	-
H 19	Hungary	YM	-	-
BG 34	Bulgaria	YM	-	-
BG 50	Bulgaria	Di	-	-
A 10	Albania	Dm	-	-
A 22	Albania	Dm	-	-
GR 2	Greece	YM	-	-
GR 6	Greece	Dm	-	-
TK 36	Turkey	Dm	-	-
TK 48	Turkey	Dm	-	-
M 1	Malta	Ds	-	-
M 2A	Malta	Ds	-	-
M 2B	Malta	Di	-	-
JOR 1	Jordan	YM	+	+
TN 11	Tunisia	Dm	-	-
TN 12	Tunisia	Dm	-	-
NIG 3	Nigeria	Ds	-	-
SA 2	S. Africa	Dm	-	-
CH 5	China	Dm	-	-

D = leaf and cane deformation
YM = yellow mosaic
VB = vein banding
s = severe
i = intermediate
m = mild
+ = presence of satRNA
- = absence of satRNA

RNA probes and hybridization conditions: Recombinant plasmids specific for satRNA of GFLV strain F13 were used and RNA probes were synthesized as described by HANS *et al.* (1992). RNA samples, denatured in MOPS formamide/formaldehyde for 10 min at 55 °C, were electrophoresed in a 1.2 % agarose denaturing gel (SAMBROOK *et al.* 1989) or spotted on nylon membranes (Hybond N+, Amersham). After electrophoresis, RNAs were transferred to Hybond N+ membranes on 10 mM sodium hydroxide 1 mM EDTA for 4 h.

Prehybridization and hybridization were as reported by HANS *et al.* (1992). Membranes were exposed for 24—48 h at -70 °C to X-ray films (Fuji-RX) using an intensifying screen.

ELISA: Direct double antibody sandwich test (DAS-ELISA), using polyclonal antibodies was performed as in FUCHS *et al.* (1991 b). Coating and conjugated-anti-

bodies were employed at 50 ng/ml and 1:20,000 dilution, respectively. Absorbance values were recorded with a Titertek Multiscan MCC/340 MK II photometer.

Results

Detection of GFLV satRNA in *C. quinoa*: Infected *C. quinoa* plants were harvested 10—12 d after inoculation, subjected to ELISA tests and total RNA was extracted. Of the 34 GFLV isolates only 5 proved to possess satRNA. The results of a dot blot assay are shown in Figure, a. The molecular weight of the different satellites detected was the same for all viral isolates, and correspond to that of GFLV-F13 satRNA (Figure, b).

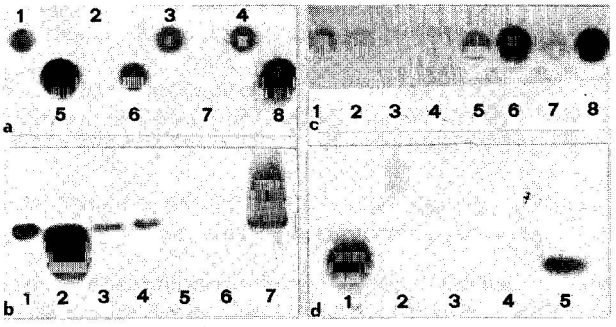

Figure: a) Dot spot hybridization of total RNA samples extracted from healthy and GFLV-infected *C. quinoa*. Samples correspond to: Mt 42 (1); Ba 6 (2); Prosecco (3); Aq 4 (4); F13 (5); Aq 7 (6); healthy (7). — b) Autoradiography of a Northern hybridization using viral and total RNA from *C. quinoa*. Lanes correspond to: total RNA, strain Aq 7 (1); viral RNA, strain F13 (2); total RNA, strain Aq 4 (3); total RNA, strain Mt 42 (4); total RNA, healthy *C. quinoa* (5); total RNA, strain Cannonau 1 A (6); total RNA strain Prosecco (7). — c) Dot spot hybridization of total RNA purified from healthy and infected grapevine leaves. Samples correspond to GFLV strains: F13 (1); Aq 4 (2); H 19 (3); healthy grapevine (4); Prosecco (5); Mt 42 (6); JOR 1 (7); Aq 7 (8). — d) Autoradiographic detection of a Northern hybridization using total RNA from healthy or infected *C. quinoa* and grapevines. Lanes correspond to: GFLV F13 from *C. quinoa* (1); GFLV JOR 1 from grapevine (2); healthy grapevine (3); healthy *C. quinoa* (4); GFLV Mt 42 from grapevine (5). — All hybridization tests were with the RNA probe described in 'Materials and methods'.

Detection of GFLV satRNA in grapevine: As shown in Figure, c, and Table, positive hybridization signals were obtained when young leaves from vines that contained virus isolates carrying satRNA were tested. Virus-free grapevines and all those whose viral isolates were negative for satRNA did not react with the probe. The RNA extraction method used (WHITE and KAPER 1989) was efficient and did not require the addition of antioxidants against phenolic compounds (NEWBURY and POSSINGHAM 1977; REZAIAN and KRAKE 1987).

Detection of satRNA was also attempted by Northern blot, but this method was not as sensitive as dot blot. In fact, satellite RNA was detected only in one grapevine sample out of five. The size of satRNA was apparently the same as that as F13 satRNA (Figure, d).

Discussion

The results of the present study show that a small RNA molecule with the same size and extensive sequence homology with the satellite RNA supported by the

French GFLV strain F13 occurs in about 15 % of *Vitis vinifera* plants naturally infected by GFLV, from Italy and Jordan. SatRNA was detected in extracts from both *C. quinoa* and *V. vinifera*, but with a lower efficiency in the latter host when Northern blot was used.

Thus, it is confirmed that, as with other nepoviruses, satRNA is not uncommon with GFLV. However, whether its presence may have a beneficial effect on the natural host by reducing the intensity of the disease remains to be established. Most of the vines containing satellite-supporting GFLV isolates (e.g. Aq 7, Aq 4, Prosecco, Mt 42, JOR 1) exhibited a symptomatology as severe as that of other vines which were deprived of satRNA.

Successful interference of satRNA with synthesis of genomic RNAs of the helper virus is likely to be proportional to the level of satRNA multiplication in the host cells. This level seems to be higher in *C. quinoa* than in *V. vinifera*, as shown by the erratic detection of satRNA by Northern blot in grapevine extracts. If so, the present results are not in contrast with the findings of FUCHS *et al.* (1991 a) relative to the modulating effect of GFLV-F13 satRNA in diseased *C. quinoa* plants, but cast doubts on the possible use of satRNA-mediated interference as a practical means for protecting grapevines from GFLV infections.

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