Replication of grapevine fanleaf virus satellite RNA transcripts in Chenopodium quinoa protoplasts

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A set of full-length cDNA clones of the satellite RNA of grapevine fanleaf nepovirus isolate F13 (GFLV-F13) was constructed with a variable number of additional, non-viral nucleotides at the 5' and 3' ends. The biological activity of the RNAs transcribed from these constructs was tested in Chenopodium quinoa protoplasts using a helper virus. When inoculated with arabis mosaic virus S (ArMV-S) RNA as helper, transcripts with 33 non-viral nucleotides at the 5' end (tr45p4) did not replicate, whereas transcripts with only one non-viral nucleotide at the 5' end (tr3S and tr3M) did replicate. Capping of the transcripts enhanced their replication. On the other hand, the presence of extra nucleotides at the 3' end had little influence on the biological activity of the in vitro transcripts. In contrast with ArMV-S, GFLV isolate 24 was not a helper for tr3M transcripts, indicating a specific interaction between the helper strain and the satellite RNA.

Introduction

Grapevine fanleaf virus (GFLV) is a widely distributed nepovirus, causing serious damage in grapes. Its genome comprises two single-stranded polyadenylated RNAs which carry a genome-linked protein at their 5' ends (Pinck et al., 1988). RNA1 (7342 nucleotides) encodes polyprotein P1 (Mr 253K; Ritzenthaler et al., 1991) and RNA2 (3774 nucleotides) encodes polyprotein P2 (Mr 122K; Serghini et al., 1990) (Fig. 1). An additional RNA of 1114 nucleotides with the same terminal structures as the genomic RNA [5' VPg and 3' poly(A)], named RNA3, is present in the F13 isolate of GFLV. This RNA is a satellite RNA because it requires the presence of a helper genome for its replication and encapsidation. RNA3 belongs to the class of large satellites (Fritsch & Mayo, 1989). It has coding capacity for a protein, P3, of Mr 37K and is translated in wheat germ extracts to produce a species of apparent Mr 39K (Pinck et al., 1988).

Except for the consensus sequence of 10 nucleotides adjacent to the VPg described by Fuchs et al. (1989 and references therein), no significant sequence homology was found between the genomic RNAs and the satellite RNA of GFLV. This consensus sequence is also present at the 5' end of other large nepovirus satellite RNAs such as the satellites of chicory yellow mottle virus (CYMV; Rubino et al., 1990), tomato black ring virus (TBRV; Hemmer et al., 1987) and a lilac isolate of arabis mosaic virus (ArMV-L; Liu et al., 1990). GFLV RNA3 and the satellite RNA of ArMV-L share 83% nucleotide sequence identity, but the homology with other nepovirus satellite RNAs is more limited. With TBRV satellite RNA, for example, only short scattered stretches of homology occur in addition to the 5' end consensus sequence (Fuchs et al., 1989). Rubino et al. (1990) noticed a short region with about 50% conservation located between nucleotides 94 and 133 in the coding region of the CYMV satellite RNA, and between nucleotides 183 and 223 in RNA3 of GFLV.

Comparison of the P3 amino acid sequences revealed 72% identity between GFLV and ArMV-L, but only...
restricted homology with TBRV (residues 345 to 371). The high content of basic residues (K and R) in the N-terminal part of P3 may indicate that this domain is involved in interactions with nucleic acids (Fuchs et al., 1989).

In this paper, we describe the construction of a set of full-length cDNA clones of the GFLV satellite RNA. Transcripts of RNA3 were synthesized using an in vitro transcription system and Chenopodium quinoa protoplasts were used to test their biological activity. We report on the importance of the 5' and 3' non-viral nucleotides for the replication of the synthetic transcripts. The ability of several virus strains to act as helper for the replication of this satellite RNA was also investigated and the specificity of the helper virus–satellite RNA association is discussed.

Methods

Viruses. GFLV and ArMV isolates were propagated on C. quinoa in a glasshouse and purified as described by Pinck et al. (1988) with the following modifications: the virus pellets from the polyethylene glycol (PEG) precipitation step and from the ultracentrifugation step were dissolved in 25 mM-glycine buffer pH 9. The virus was further purified and the RNAs were extracted as described by Pinck et al. (1991).

Bacterial strains. Escherichia coli strains JM109 and TG2 were transformed as described by Hanahan (1983) or by electroporation (Sambrook et al., 1989). All constructs were cloned in the phagemid BlueScribe M13+ (pBS +, Stratagene).

Construction of a full-length GFLV RNA3 cDNA. Clone pA4 containing the cDNA of GFLV RNA3 inserted in a pUC9 vector was described by Fuchs et al. (1989). From this clone, the cDNA of RNA3 was excised by digestion with HindIII and EcoRI and cloned into the pBS + expression vector cut with the same enzymes. The resulting copy of RNA3 contained 33 non-viral nucleotides at the 5' end and 22 non-viral nucleotides at the 3' end arising from the cloning strategy. Positive-sense RNA synthesis was under control of the T3 promoter (Fig. 2). The transcription vector was named p45p4 (Fuchs, 1989).

Site-directed mutagenesis

(i) Elimination of the 5' non-viral nucleotides. The non-viral nucleotides located between the T3 promoter and the first nucleotide of RNA3 were deleted by mutagenesis with a synthetic oligonucleotide complementary to nucleotides 1 to 20 of RNA3 and to the 12' terminal nucleotides of the T3 promoter region: 5' GTCCTAGAAATTTTTCTACATTAGTGAGGG 3'. The mutation procedure was carried out according to the manufacturer’s instructions (Stratagene). Recombinant clones were recovered by hybridization on Colony/Plaque Screen membranes using 32P-labelled mutagenic oligonucleotide, by colony hybridization using 32P-labelled mutagenic oligonucleotide, by restriction analysis and by nucleotide sequence analysis as described above. This transcription vector was named p3M (Fig. 2).

(ii) Elimination of the 3' non-viral nucleotides. The 22 non-viral nucleotides at the 3' end of plasmid p3S were substituted by a HindIII site so as to generate a cDNA of GFLV RNA3 with three extra nucleotides at the 3' end. The synthetic oligonucleotide used was 5' TATAGGGCGAATTCAAGC(T21) 3', complementary to the poly(A) tail of the cDNA of RNA3 and to 17 nucleotides of pBS + located after the cDNA of RNA3. The mutagenesis was performed using the procedure for efficient selection of mutated DNA developed by Kunkel (1985). Recombinant DNA molecules were screened by colony hybridization using 32P-labelled mutagenic oligonucleotide, by restriction analysis and by nucleotide sequence analysis as described above. This transcription vector was named p3S (Fig. 2).

In vitro transcription. DNA was linearized by appropriate enzymes, phenol–chloroform-extracted and ethanol-purified. For uncapped transcripts, 10 μg of linearized DNA was incubated in 100 μl containing 40 mM-Tris–HCl pH 7.5, 6 mM-MgCl₂, 2-2 mM- spermidine, 0.4 mM each of rATP, rGTP, rCTP and rUTP, 1-6 units/μl RNasin and 2 units/μl T3 RNA polymerase for 1 h at 37 °C. For synthesis of capped transcripts, GTP was decreased to 25 μM and 500 μM-cap analogue m7GpppG was added. After 30 min at 37 °C, the GTP concentration was adjusted to 0.4 mM and the reaction continued for 1 h. The DNA was removed by treatment with RNase-free DNase I for 30 min at 37 °C. The transcripts were purified by phenol–chloroform and other extractions followed by ethanol precipitation. The size and integrity of transcripts were checked by electrophoresis under denaturing conditions in a 1% formaldehyde-agarose gel. By convention, the transcripts derived from p45p4, p3S and p3M were named tr45p4, tr3S and tr3M, respectively.

In vitro translation. The coding capacity of the transcripts was tested in a wheat germ system prepared as described by Godfrey-Colburn et al. (1985) and in the nuclease-treated rabbit reticulocyte lysate system (NT-RRLS) obtained from Promega, as described by Margis et al. (1991). The 35S-labelled proteins synthesized were analysed on a 15% SDS–polyacrylamide gel using the system of Laemmli (1970).

C. quinoa protoplast preparation and inoculation.

(i) Preparation. C. quinoa plantlets were conditioned in a growth chamber at 22 °C during a 16 h light period and at 18 °C during the night. The light was provided by a sodium lamp producing 8000 lux. Plants were regularly watered with a 1/1000 (v/v) solution of Lifan (Bayer). After 10 to 15 days, plants with four to six pairs of leaves were used for protoplast isolation. Leaves were rinsed briefly in a 70% ethanol bath, sterilized in a 1% bleach bath for 10 min and washed three times in water for 10 min per wash. They were cut into small squares and placed in 0-6 M-manitol, 10 mM-CaCl₂, pH 5-6. The leaf fragments were digested overnight at 25 °C with 1-3% cellulase, 0-3% Macerozyme (R-10, Onozuka) and 0-1% BSA in culture medium containing 0-2 mM-KH₂PO₄, 1 mM-KNO₃, 1 mM-MgSO₄, 10 mM- CaCl₂, 1 μM-KI, 0-01 mM-CuSO₄, pH 5-6 (Rottier et al., 1979). Protoplasts were freed from tissue debris on a 100 μm filter, washed twice with 0-54 m-sucrose and three times with 0-6 M-manitol, pH 5-6. Fluorescein diacetate (FDA) staining was used to determine the percentage of viable protoplasts (Widholm, 1972).
(ii) PEG inoculation. C. quinoa protoplasts were inoculated according to the procedure of de Varennes et al. (1984). Protoplasts (1 × 10^9) concentrated in 100 μl 0.6 M-mannitol pH 5.6 were mixed with 250 μl 30% PEG-6000, 3 mM-CaCl_2, 0.4 M-mannitol, containing the RNA inoculum. The suspension was gently mixed for 15 s, diluted with 2.5 ml 0.6 M-mannitol, 10 mM-CaCl_2, pH 5.6, kept on ice for 10 min and washed three times with 0.6 M-mannitol, 10 mM-CaCl_2, pH 5.6. The protoplasts were adjusted to 3 × 10^9 pm c/ml culture medium and were incubated in small Petri dishes with a bottom layer of 1% agarose (Seaplak LE, Seakem) containing culture medium at 22 °C under continuous diffuse lighting. In some experiments, 30 μC [35S]methionine per 1 × 10^9 protoplasts was added to the culture medium for labelling.

(iii) Electroporation procedure for inoculation. Protoplasts were prepared as described above but the digestion time was reduced to 3.5 h, the temperature increased to 30 °C and the sucrose wash was omitted. The protoplasts were infected as described by Veidt et al. (1992). After the isolation step, protoplasts were kept overnight at 4 °C. Genomic viral helper RNA supplemented or not with the synthetic transcripts was added to 2 × 10^8 protoplasts in 0.5 ml 0.6 M mannitol, 0.1 mM-CaCl_2, pH 5.6. The suspension was transferred to a cold 0.4 cm path-length cuvette and electroporated during a 12 ms high voltage pulse provided by discharge of a 125 kV capacitor (Bio-Rad) set to 300 V. Protoplasts were incubated for 30 min on ice, washed once with 0.6 M-mannitol, 0.1 mM-CaCl_2, pH 5.6, and cultivated as described for PEG inoculation, except that 0.3 mg/ml carbamycin was added to the culture medium.

Detection of viral RNAs in total RNA from infected protoplasts. Antisense RNA probes (riboprobes) specific for the GFLV genome were used to detect the presence of GFLV RNA synthesized in protoplasts. The PstI–EcoRI fragment of cDNA clone pA47 of RNA1 (nucleotides 2176 to 3100), the EcoRI–HindIII fragment of cDNA clone pG38 of RNA2 (nucleotides 147 to 1020) and the SphI–SalI fragment of cDNA clone pA5 of RNA3 (nucleotides 129 to 753) were subcloned into compatible sites of PBS+. The resulting plasmid, pSI, pSII and pSIII, respectively, were used for the synthesis of the corresponding SI, SII and SIII riboprobes. pSI was linearized by HindIII digestion and transcribed by T3 RNA polymerase. pSII and pSIII were linearized by EcoRI treatment and transcribed by T7 RNA polymerase. Transcription was performed on 1 μg of template DNA as described above except that rUTP was replaced by 60 μCi [α-32P]UTP.

Protoplasts were harvested at various times post-inoculation, pelleted and disrupted in 200 μl 50 mM-Tris–HCl pH 7.5, 100 mM-NaCl, 1 mM-EDTA, 1% SDS. Total nucleic acid was twice extracted with phenol–chloroform (1:1, v/v) and ethanol-precipitated in the presence of 0.3 M-sodium acetate pH 5.2. To recover RNA free of DNA, the pellet was twice washed with 3 M-sodium acetate pH 5.2. Total protoplast RNAs were separated by electrophoresis on a 1% formaldehyde–agarose gel and blotted for 3 h with 10 mM-NaOH and 1 mM-EDTA to a Hybond N+ membrane.

Hybond N+ membrane was prehybridized for 1 to 4 h in 50% formamide, 0.5% non-fat milk, 1 × SSPE, 1% SDS, 200 μg/ml yeast RNA and 500 μg/ml sonicated salmon sperm DNA. After addition of the labelled probes, hybridization was continued overnight at 50 °C. Membranes were washed twice for 15 min in 2 × SSPE, 0.1% SDS and for 15 min in 0.5 × SSPE, 0.1% SDS at 50 °C.

Immunoprecipitation. To detect the coat protein of GFLV, healthy and infected C. quinoa protoplasts were cultured in the presence of 30 μCi [35S]methionine per 1 × 10^9 protoplasts and harvested at various times post-inoculation. The coat protein was detected by immunoprecipitation as described by Demangeat et al. (1990) in aliquots containing 1 × 10^5 protoplasts.

### Results and Discussion

#### Transcription of full-length GFLV RNA3 cDNA

The transcript tr45p4 produced by in vitro run-off transcription of EcoRI-linearized p45p4 using RNA polymerase T3 contained 33 and 22 non-viral nucleotides at the 5' and 3' ends, respectively (Fig. 2). To determine the influence of non-viral nucleotides on infectivity of tr45p4, the non-viral nucleotides at the 5' end were first eliminated by site-directed mutagenesis to give clone p3S. The transcript tr3S obtained from this clone contained only one non-viral G nucleotide at the 5' end (nucleotide +1) and 22 non-viral nucleotides at the 3' end.

The plasmid p3M was constructed by substitution of a HindIII site for the 22 non-viral nucleotides at the 3' end of clone p3S. The transcript tr3M obtained from HindIII-linearized p3M differed from natural RNA3 only in the absence of VPg and the presence of one non-viral G at the 5' end and three non-viral residues at the 3' end of the poly(A) tail.

The yield of transcript tr45p4 was fivefold that of tr3S and tr3M presumably because the T3 promoter in p45p4 contained two Gs at its 5' terminus, whereas tr3S and tr3M contained only one G (Fig. 2). It is known that the efficiency of in vitro transcription with such a system is affected by the context near the transcription initiation site (Eggen et al., 1989). In all cases, the yield of capped transcripts was twofold lower than the yield of uncapped transcripts (data not shown).

#### In vitro translation of RNA3 transcripts

The messenger properties of tr45p4, tr3S and tr3M were analysed in wheat germ extracts and in NT-RLS. The proteins translated from all transcripts comigrated with the P3 protein synthesised from the natural RNA3 (data not shown) (Pinck et al., 1988). This indicated that the full-length clones of RNA3 were not altered during the cloning and mutagenesis steps.

#### Replication of GFLV RNA in C. quinoa protoplasts

A yield of 2 × 10^6 to 4 × 10^6 C. quinoa protoplasts per g of leaves was regularly obtained. FDA staining indicated that 98 to 100% of the protoplasts were viable after the isolation step and 50 to 60% after PEG treatment. Eighty and 60% of the protoplasts survived after 48 h and 72 h of culture, respectively. Similar protoplast viability was obtained by de Varennes et al. (1984).

The capacity of freshly prepared C. quinoa protoplasts to replicate GFLV RNA was tested after infection by the PEG inoculation method. Protoplasts (1 × 10^6) were
In order to determine the minimum level of RNA inoculum necessary for infection, $1 \times 10^6$ protoplasts were infected with increasing amounts of GFLV RNA by using the PEG method. Total RNAs from $1 \times 10^5$ protoplasts were extracted 72 h post-inoculation and the presence of viral RNA was detected by primer extension (Dore et al., 1989) using the Pr1 primer complementary to nucleotides 77 to 94 of RNA1 (Fig. 1). Protoplasts inoculated with $1 \mu g$ of GFLV RNA were not infected, whereas protoplasts inoculated with 5 or $10 \mu g$ of viral RNA were infected (data not shown). In all further infection experiments, the ratio of $10 \mu g$ GFLV RNA per $1 \times 10^6$ protoplasts was used for infection assays.

**Replication of the RNA3 transcripts in C. quinoa protoplasts**

Experiments to demonstrate the biological activity of the RNA3 transcripts *in vitro* were performed in *C. quinoa* protoplasts inoculated by the PEG method with ArMV-S, an isolate free of satellite RNA and originating from grapevine (Fuchs et al., 1991), as helper because no satellite-free GFLV was available as shown later. Protoplasts ($5 \times 10^5$) were inoculated with $5 \mu g$ ArMV-S, supplemented with uncapped and capped tr45p4, tr3S and tr3M obtained from 20 $\mu g$ of the corresponding linearized DNA. Total RNAs from $5 \times 10^5$ protoplasts were extracted 72 h post-inoculation and tested for the presence of satellite RNA using the SIII riboprobe. The signal corresponding to RNA3 was very faint after a 12 day autoradiographic exposure of the membrane, indicating only very low replication of the uncapped and capped tr45p4 transcripts (data not shown). Similarly, the uncapped and capped tr3S and tr3M transcripts were poorly replicated. These results suggested that the inoculation method used was responsible for the low efficiency obtained. To improve the inoculation efficiency the electroporation method was used: $2 \times 10^5$ protoplasts were electroporated with $2 \mu g$ ArMV-S RNA as a helper, supplemented with uncapped and capped tr45p4, tr3S and tr3M. Total RNAs were extracted from $1 \times 10^5$ protoplasts 72 h post-inoculation and analysed with the three riboprobes previously used for GFLV RNA detection. Under these conditions the detection signal corresponding to ArMV-S RNA2 was higher than for RNA1 (Fig. 5, lanes 2 to 7); this is related to the large amount of RNA2 in ArMV-S and to the similarity of the SII probe with the sequence of ArMV-S RNA2 (unpublished results). In contrast with the previous results, unambiguous signals corresponding to RNA3 were visible after an overnight exposure. The reduced loss of protoplasts after electroporation (approx. 10%) and the rapidity of the infection step resulting in limited degradation of the inoculated RNA may explain the

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Fig. 3. Northern blot analysis of the time course of GFLV RNA synthesis in protoplasts inoculated with GFLV RNA. Protoplasts ($1 \times 10^6$) were inoculated with $10 \mu g$ RNA by the PEG method. RNAs were extracted from $1 \times 10^5$ protoplasts at 0 (lane 3), 12 (lane 4), 24 (lane 5), 48 (lane 6), 72 (lane 7) and 96 (lane 8) h post-inoculation. Total protoplast RNAs were separated by electrophoresis on a 1% formaldehyde-agarose gel, blotted on a Hybond N+ membrane and hybridized with a mixture of the three $32^P$-labelled riboprobes specific for each GFLV RNA. Lanes 1 and 9, GFLV RNA as control; lane 2, RNAs from healthy protoplasts.

Fig. 4. Detection of the 56K coat protein in $1 \times 10^6$ protoplasts infected with $10 \mu g$ GFLV RNA using the PEG method. Immunoprecipitation was carried out on total proteins of $1 \times 10^5$ protoplasts with anti-viral serum diluted 1/1000, at 0 (lane 2), 12 (lane 3), 24 (lane 4), 48 (lane 5), 72 (lane 6) and 96 (lane 7) h post-inoculation. The immunoprecipitates were analysed on a 10% SDS-polyacrylamide gel. Lane 1, uninfected protoplasts as control.
transcript infectivity, but their presence at the 3' end is less inhibitory. An inhibitory effect of 5' non-viral extensions has also been demonstrated for synthetic nucleotides at the 5' end has a dramatic effect on detected in each case. Thus, the presence of extra satellite RNA transcripts of TBRV (Greif et al., 1990) RNA3 signal is compared to the amount of RNA2 that the uncapped tr45p4 transcript was able to replicate non-viral nucleotides at the 3' end was markedly more (Fig. 5, lanes 4 and 5). The transcript tr3M with three and 22 non-viral nucleotides at the 3' end was infectious and cucumber mosaic virus (CMV; Masuta 2002) is not infectious when capped. Similar results were also obtained for cowpea mosaic virus (Vos et al., 1988), barley yellow dwarf virus (Young et al., 1991) and beet western yellows virus (Veidt et al., 1992). Although the role of the cap is not precisely known, it probably protects the natural or synthetic RNAs from degradation by exonucleases and increases their biological activity (Shimotohno et al., 1992). It is also possible that capped transcripts are more efficiently translated immediately after inoculation if the P3 protein somehow plays a role in the replication of the satellite RNA.

Ability of different nepovirus strains to act as helper

Two GFLV isolates were expected to be potential helpers, the GFLV-Tu strain shown to be free of satellite RNA when multiplied on C. quinoa (Fuchs et al., 1991) and the GFLV-24 strain originating from Malta, kindly provided by Professor G. P. Martelli (Bari, Italy) which was also free of satellite. Both isolates are serologically related to GFLV-F13. Each was tested for its ability to replicate tr3M, ArMV-S being used as control. In these experiments, the same batch of protoplasts, the same preparation of capped tr3M and the same ratio of tr3M helper RNA were used for the infection.

A control experiment revealed that when GFLV-Tu RNA which contained no RNA3 detectable with the SIII riboprobe was used alone to inoculate C. quinoa protoplasts, an RNA nearly comigrating with the natural GFLV-F13 RNA3 (Fig. 6, lane 8) was detected which would interfere with detection of the RNA derived from the inoculated transcript (Fig. 6, lane 9), rendering this strain unusable as a helper. The presence of this presumed satellite RNA was unexpected since it was not detected with cDNA probes in GFLV-Tu-infected plants (Fuchs et al., 1991). Valverde et al. (1991) reported better performance of electroporation compared with the PEG inoculation method.

As noticed previously after PEG-mediated infection, capped and uncapped tr45p4 were not infectious in electroporated protoplasts (Fig. 5, lanes 2 and 3). This is in contrast to the results of Liu et al. (1991) who demonstrated that, in C. quinoa plants, replication of the synthetic transcripts of ArMV-L satellite RNA was not inhibited despite the presence of six or 29 non-viral nucleotides at the 5' end. Similarly, Fuchs (1989) found that the uncapped tr45p4 transcript was able to replicate in C. quinoa plants. The failure of tr45p4 to replicate in C. quinoa protoplasts may be due to the limited number of sites present in protoplasts available for successful initiation of infection.

The transcript tr3S with one non-viral G at the 5' end and 22 non-viral nucleotides at the 3' end was infectious (Fig. 5, lanes 4 and 5). The transcript tr3M with three non-viral nucleotides at the 3' end was markedly more infectious than tr3S (Fig. 5, lanes 6 and 7) when the RNA3 signal is compared to the amount of RNA2 detected in each case. Thus, the presence of extra nucleotides at the 5' end has a dramatic effect on transcript infectivity, but their presence at the 3' end is less inhibitory. An inhibitory effect of 5' non-viral extensions has also been demonstrated for synthetic satellite RNA transcripts of TBRV (Greif et al., 1990) and cucumber mosaic virus (CMV; Masuta et al., 1988) and for synthetic transcripts of genomic RNA from several animal and plant viruses (van der Werf et al., 1986; Dawson et al., 1986; Janda et al., 1987; Ziegler-Graff et al., 1988, Heaton et al., 1989).
Fig. 6. Northern blot showing the replication of tr3M using different genomic viral RNAs as helper. Protoplasts (2 \times 10^5) were electroporated with 2 \mu g GFLV-24 RNA (lane 4), 2 \mu g GFLV-24 RNA plus 4 \mu g tr3M (lane 5), 2 \mu g ArMV-S RNA (lane 6), 2 \mu g ArMV-S RNA plus 4 \mu g Tr3M (lane 7), 2 \mu g GFLV-Tu RNA (lane 8), 2 \mu g GFLV-Tu RNA plus 4 \mu g tr3M (lane 9). Lane 1, GFLV RNA as control; lane 2, healthy protoplasts; lane 3, 2 \times 10^5 protoplasts inoculated with 10 lag GFLV RNA. Northern blot analysis was performed as described in the legend to Fig. 3.

the reappearance of a satellite of the tobacco mosaic virus U5 strain (TMV-U5) after several passages through Nicotiana tabacum for some subcultures of TMV-U5 which were initially free of detectable satellite but which had been derived from isolates containing satellite RNA. A similar situation may occur with GFLV-Tu. It is also possible that satellite RNA multiplication in protoplasts is more efficient than in whole plant cells.

When GFLV-24 genomic RNAs were used as helper, no replication of the transcripts was detected 72 h post-inoculation (Fig. 6, lane 5). Thus, although GFLV-24 is serologically related to GFLV, it cannot replicate the RNA3 transcripts of GFLV-F13. Liu et al. (1991) demonstrated that GFLV was not a helper for ArMV-S satellite RNA; they also showed that the ivy and ash isolates of ArMV were not helpers for ArMV-L satellite RNA in contrast to hop and sugar-beet isolates of the same virus. Similarly, TBRV satellite RNA from serotypes S or G multiplied only with TBRV of the same serotype (Murant & Mayo, 1982). These results illustrate the close relationship between a satellite RNA and its helper virus.

When protoplasts were electroporated with ArMV-S RNA and tr3M, F13 satellite RNA was detected 72 h post-inoculation. The ArMV-S helper RNAs were able to replicate tr3M in spite of the low homology of the RNA sequence between ArMV-S and GFLV-F13, indicated by the low rate of hybridization of the ArMV genomic RNA with the SI and SII riboprobes (Fig. 6, lane 7). It has been demonstrated for TBRV that the capacity to replicate its satellite depends only on the origin of RNA1 (Murant & Mayo, 1982). Using pseudorecombinants constructed from the Fny-CMV and Sny-CMV strains, Roosink & Palukaitis (1991) mapped the ability to support the replication of the WL1-CMV satellite RNA in zucchini plants to RNA1. In our case, it would be interesting to construct chimeric infectious synthetic RNA1 transcripts containing sequences of GFLV-F13, GFLV-24 and ArMV-S RNA1. Their capacity to replicate tr3M in C. quinoa protoplasts should permit mapping of the sequences involved in the interactions between RNA1 and the satellite of GFLV-F13. The development of a C. quinoa protoplast system and the obtaining of infectious transcripts will greatly help investigations on the mechanisms of satellite RNA replication, the helper virus–satellite association and the role of the P3 protein.

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References


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