Replication and encapsidation of the viroid-like satellite RNA of lucerne transient streak virus are supported in divergent hosts by cocksfoot mottle virus and turnip rosette virus

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Cocksfoot mottle sobemovirus supports replication and encapsidation of the viroid-like satellite RNA (sat-RNA) of lucerne transient streak virus (LTSV) in two monocotyledonous species, Triticum aestivum and Dactylis glomerata. Additionally, LTSV sat-RNA replicates effectively in the presence of turnip rosette sobemovirus in Brassica rapa, Raphanus raphanistrum and Sinapis arvensis, but not in Thlaspi arvense or Nicotiana bigelovii, indicating that host species markedly influence this interaction. Previous reports of the association between LTSV sat-RNA and helper sobemoviruses were limited to dicotyledonous hosts. Our results demonstrate that the biological interaction between these two entities spans divergent dicotyledonous and monocotyledonous species.

Lucerne transient streak virus (LTSV) occurs in Australia (Blackstock, 1978), New Zealand (Forster & Jones, 1979) and Canada (Paliwal, 1983). In addition to a 4.5 kb single-stranded genomic RNA (RNA-1), LTSV encapsidates a viroid-like satellite (sat) RNA (RNA-2) that is dependent upon the helper virus (i.e. LTSV) for replication (Tien-Po et al., 1981; Jones et al., 1983). Viroid-like sat-RNAs have also been reported in a few other sobemoviruses, namely velvet tobacco mottle virus (VTMoV; Randles et al., 1981; Francki et al., 1986), Solanum nodiflorum mottle virus (SNMV; Gould & Hatta, 1981; Jones & Mayo, 1984) and subterranean clover mottle virus (SCMoV; Francki et al., 1983a). Most sobemoviruses including cocksfoot mottle virus (CiMV), turnip rosette virus (TRoSV), southern bean mosaic virus (SBMV) and sowbane mosaic virus (SoMV), however, are normally devoid of sat-RNAs (reviewed by Hull, 1988). The RNA-2 of LTSV lacks helper specificity in that it replicates in association with SoMV (Francki et al., 1983b) and SBMV (Paliwal, 1984b). Moreover, preliminary data (Jones & Mayo, 1984) indicate that TRoSV may support LTSV RNA-2 replication in Chenopodium amaranticolor. Previous studies (reviewed by Fritsch & Mayo, 1989) concerning propagation of viroid-like sat-RNAs by helper viruses had been limited to dicotyledonous hosts, and only recently (Miller et al., 1991) has a sat-RNA been shown to be associated with barley yellow dwarf luteovirus, a virus that affects monocotyledons. We report that LTSV RNA-2 effectively replicates in monocotyledonous species in the presence of CfMV, and is packaged in CfMV virions. Furthermore, we report that replication of LTSV RNA-2 in the presence of TRoSV is host-dependent.

CiMV was propagated in Triticum aestivum cv. Hatton, LTSV (Canadian isolate, LTSV-Ca) in Trigonella foenum-graecum and TRoSV in Brassica rapa and Nicotiana bigelovii. LTSV RNA-2 was purified according to Paliwal (1984b) and was established to be free of LTSV RNA-1. CfMV and LTSV RNA-2 were co-inoculated onto T. aestivum and Dactylis glomerata (cultures hereafter referred to as CfMV-L2) as a mixture of 500 μg of virions and 200 μg of RNA per ml. Similarly, TRoSV virions and LTSV RNA-2 were coinoculated (referred to as TRoS-L2) to B. rapa, Raphanus raphanistrum, Sinapis arvensis, Thlaspi arvense and N. bigelovii.

Virions were purified by the following procedure. Systemically invaded leaves, harvested 4 weeks post-infection and frozen at -60 °C, were thawed in 200 mM-sodium acetate-acetic acid buffer pH 5.0, homogenized in a Waring blender and the homogenate was strained through cheese-cloth. The pH of the extract was adjusted to 5.0 with 1 M-acetic acid and maintained at 25 °C for 90 min. Following the addition of 0.5 vol. chloroform plus butanol (75:25 v/v), the extract was stirred on ice for 15 min and then centrifuged at 8000 g for 20 min. Virions
in the aqueous phase were sedimented by ultracentrifugation (105,000 g for 2.5 h) through a 15% sucrose cushion and then suspended in 10 mM-sodium phosphate buffer pH 7.0 containing 100 mM-sodium chloride and 0.025% sodium azide. Following two additional cycles of differential centrifugation, virions were stored at 5 °C.

Isolation of viral RNAs and electrophoresis in 3-5% polyacrylamide gels were according to Paliwal (1984a). U.v. densitometer scans of electrophoresed total virion RNAs showed that, in addition to viral genomic RNA (RNA-1), another small RNA moiety (RNA-2) was apparent in the following preparations: LTSV (Fig. 1 a); CfMV-L2 that had been propagated in T. aestivum and D. glomerata (Fig. 1 c and d); and TRosV-L2 from R. raphanistrum and S. arvensis (Fig. 1 g and h). The migration pattern of the small RNA moiety from CfMV-L2 and TRosV-L2 was indistinguishable from that of LTSV RNA-2. There was no evidence for the presence of the smaller RNA moiety in virion RNA preparations from CfMV or TRosV (Fig. 1 b and e) or those from TRosV-L2 recovered from T. arvense (Fig. 1 i) or N. bigelovii (Fig. 1 f). It is significant that although LTSV RNA-2 replicated in plant species belonging to the families Brassicaceae and Gramineae in the presence of TRosV and CfMV, respectively, these are not hosts for LTSV (Hull, 1988).

Nucleic acid hybridization has proven to be a reliable method for detecting a number of viroids (reviewed by McInnes & Symons, 1991). To identify the presence of LTSV sat-RNA sequences in the co-inoculated viral cultures, dot-blot hybridizations were performed as follows. Purified virions were spotted in duplicate directly onto two nitrocellulose filters using a Bio-Rad Bio-Dot filtration apparatus as described by Waterhouse et al. (1986). The filters were treated in solutions of 10% SDS and 3×SSC buffer (1×SSC contains 150 mM-NaCl, 15 mM-sodium citrate pH 7.0) according to Bijaisoradat & Kuhn (1988) and hybridized overnight at 55 °C as described by Ivanov & Gigova (1986). One of the filters (Fig. 2 a) was hybridized with a 32P-labelled (by the random primed DNA polymerase reaction) full-length
cDNA clone of LTSV-Ca RNA-2 (AbouHaidar & Paliwal, 1988) and the second (Fig. 2b) with a cDNA clone of LTSV RNA-1 (unpublished), labelled by the same method. The specific radioactivity of both probes was about 10^6 c.p.m./µg of DNA and the concentration of the labelled probes in the hybridization mixtures was 1.7 x 10^6 c.p.m./ml. The results presented in Fig. 2(a) show that positive hybridization signals with the probe specific for LTSV RNA-2 were obtained with CfMV-L2 propagated in T. aestivum, and TRosV-L2 recovered from S. arvensis and B. rapa. No hybridization was detected with CfMV, TRosV, TRosV-L2 propagated in N. bigelovii, nor with virions of TRosV-L2 initially propagated in N. bigelovii and subsequently back-inoculated to B. rapa (Fig. 2a). This result indicates that LTSV RNA-2 does not replicate in N. bigelovii. The dot-blot hybridizations with the probe specific for LTSV RNA-1 (Fig. 2b) did not show any positive reaction with the CfMV-L2 or TRosV-L2 samples, suggesting that the viral preparations used in this study were not contaminated with LTSV. These results also indicate the absence of any significant sequence homology between the genomic RNA of LTSV and those of CfMV or TRosV (Fig. 2b). In situ hybridization of viral RNA samples in dried 2% agarose-formaldehyde gels showed that the high M_r genomic RNAs from LTSV, CfMV and TRosV did not hybridize with the LTSV RNA-2 specific probe, suggesting that no sequence homology exists between these RNAs and LTSV sat-RNA (data not shown).

Jones & Mayo (1984) observed that co-inoculation of TRosV and LTSV RNA-2 on Chenopodium quinoa resulted in an apparent change of lesion type, from chlorotic to necrotic, and this was considered to be due to LTSV RNA-2 replication in the presence of TRosV. A small proportion of the progeny virus from this mixed infection, following propagation in B. rapa, induced necrotic lesions on C. amaranticolor. This indicated that LTSV RNA-2 may, to some extent, be encapsidated by TRosV virions. These results were equivocal, however, because the necrotic lesions were few in number and/or difficult to detect, and symptoms were occasionally erratic. The work presented here provides evidence, via nucleic acid hybridization, that both TRosV and CfMV support the replication and packaging of LTSV RNA-2, confirming that this sat-RNA does not specifically require genomic LTSV RNA for replication (Paliwal, 1984b; Jones & Mayo, 1984). Other instances of nonspecificity in the interaction between helper sobemoviruses and sat-RNA have been reported. For example, LTSV RNA-1 supports the replication of RNA-2 of both SNMV (Jones & Mayo, 1983b) and SCMoV (Keese et al., 1983; Dall et al., 1990). Interestingly, Davies et al. (1990) found two structurally different sat-RNAs associated with a single SCMoV isolate. Conversely, helper–satellite specificity has also been reported for some of the satellite-harbouring sobemoviruses: SNMV will not support the replication of RNA-2 of VTMoV (Gould et al., 1981) or LTSV (Jones & Mayo, 1984); SNMV RNA-2 does not replicate in the presence of VTMoV (Gould et al., 1981); and although SoMV supports RNA-2 of LTSV, it is not a suitable helper for the sat-RNAs from VTMoV (Gould et al., 1981) or SCMoV (Franck et al., 1983b).

In the present study we observed that TRosV supports LTSV RNA-2 replication in B. rapa, R. raphanistrum and S. arvensis, but not in T. arvense or N. bigelovii. Paliwal (1984b) reported that LTSV RNA-2 replicated efficiently in the presence of SBMV in T. foenum-graecum, but poorly in Phaseolus vulgaris cv. Bountiful. Similarly, SBMV supports LTSV RNA-2 replication in Trifolium incarnatum but not in Glycine max (Y.C. Paliwal, unpublished data). RNA-2 replication presumably depends on the helper virus RNA polymerase; our results, however, indicate that satellite RNA (RNA-2) replication may depend not only on the helper virus but also on the host plant. For instance, specific host factors may be required for the helper virus–sat-RNA interaction. The requirements for replication of the helper virus and those of the sat-RNA may not be the same. The nature of this mechanism remains to be determined.

The host species in which helper sobemoviruses have been shown previously to support LTSV RNA-2 replication are limited to members of the dicotyledonous families, namely Leguminosae, Chenopodiaceae and Solanaceae. The present study extends the host range of these biologically interacting entities to include the dicotyledonous family Brassicaceae. Furthermore, it demonstrates that LTSV sat-RNA replicates effectively and is encapsidated in the presence of CfMV in two monocotyledonous species of the family Gramineae. Obviously, a viroid-like sat-RNA (i.e. LTSV RNA-2) may interact with a suitable helper virus in divergent dicotyledonous and monocotyledonous species. Thus it is of added interest to elucidate this interaction, especially with respect to the mode of origin of LTSV sat-RNA.

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References


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