Differential Interactions of Bamboo Mosaic Potexvirus Satellite RNAs, Helper Virus, and Host Plants

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Bamboo mosaic potexvirus (BaMV) supports the replication and encapsidation of a single-stranded satellite RNA (satBaMV). Full-length infectious cDNA clones of sat-BaMV variants with a 0.7 to 7.5% difference in nucleotide sequence were constructed, and in vitro transcripts were co-inoculated with BaMV RNA to barley protoplasts. Co-inoculation with BSL6 satBaMV transcripts resulted in a greatly reduced accumulation of genomic and subgenomic RNAs whereas co-inoculation with the prototype BSF4 or other variants did not. satBaMV variant BSL6-mediated interference occurred at the genomic RNA, subgenomic RNA, and capsid protein levels whereas the synthesis of minus strand was not significantly affected. Attenuation of the BaMV symptoms also was observed in *Chenopodium quinoa* and *Nicotiana benthamiana* plants co-inoculated with BSL6 satBaMV. In protoplasts of barley and *N. benthamiana*, BSF4 and BSL6 variants replicated at a similar level whereas in the inoculated leaves of *C. quinoa* and *N. benthamiana* BSL6 accumulated to a much lower level than BSF4, and was encapsidated. BSL6 satBaMV was rarely detected in the systemic leaves of *N. benthamiana* co-inoculated with BaMV RNA, indicating that BSL6 satBaMV may be a movement-inefficient variant. It is suggested that this variant may provide a potential source for both resistance against BaMV and insights into the interactions of satellite RNA, helper virus, and symptom expression.

Satellite RNAs (sat RNAs) are subviral agents that replicate in hosts only in the presence of a helper virus. They share no significant sequence homology with the helper viruses and are dependent on the helper for encapsidation, replication, and movement (Murphy et al. 1995). They have attracted considerable attention because some sat RNAs can interfere with helper virus replication and/or attenuate virus-induced symptoms. sat RNAs can be used as biocontrol agents against their helper viruses, when they are inoculated to or present as a transgene in the host plants (Tien and Wu 1991).

Of the known single-stranded (ss) sat RNAs, most with genomes less than 0.7 kb do not encode any detectable proteins in vivo but some of them can substantially reduce virus accu-

mulation and modify virus-induced symptoms (Roossinck et al. 1992; Murphy et al. 1995). On the other hand, few sat ssRNAs with genomes larger than 0.7 kb are capable of encoding a non-structural protein. These mRNA-type sat RNAs usually do not affect the amount of helper virus accumulation and cause little or no alternation of virus symptoms (Fritsch et al. 1993). Symptoms caused by arabis mosaic virus (ArMV) were found to be either exacerbated or ameliorated by the presence of its associated sat RNA (Liu et al. 1991). Likewise, the symptom expression in groundnut rosette virus (GRV)-infected groundnut plants is greatly influenced by the presence of GRV sat RNA (Murant and Kumar 1990). One sat variant was found to down-regulate the replication of GRV (Taliensky and Robinson 1997).

Bamboo mosaic virus (BaMV), a member of the potexvirus group, contains an ss, positive-sense RNA genome with five conserved open reading frames (ORFs) (Lin et al. 1994; Yang et al. 1997). sat RNA of BaMV (satBaMV) is the only example of sat RNA in association with the potexvirus group reported (Lin and Hsu 1994). satBaMV contains an ORF for a protein of 20 kDa (P20) that can be detected both in in vitro translation systems (Lin and Hsu 1994) and in infected cells (N.-S. Lin, *unpublished*). However, unlike other mRNA-type sat RNAs, the satBaMV-encoded P20 is not essential for sat-BaMV replication (Lin et al. 1996).

Variants of satBaMV from different sources showed a nucleotide sequence variation between 0.7 and 7.5% (Liu et al. 1997). The phylogeny of satBaMVs does not correspond to the taxonomic relationship of the bamboo hosts from which they were first isolated nor does it correspond to the locations from where they were collected (Liu et al. 1997). In this study, we prepared the infectious in vitro transcripts from cDNA clones of seven satBaMV variants and tested their biological activities. In contrast to the previously reported BSF4 sat-BaMV, which does not significantly affect BaMV replication (Lin et al. 1996), transcripts of the variant pBSL6 markedly reduced the accumulation of BaMV genomic RNA in the infected protoplasts. Comparative effects of BSF4 and BSL6 satBaMVs on BaMV accumulation and symptom modulation in the infected plants are also reported.

RESULTS

Effects of satBaMV variants on BaMV genomic RNA accumulation in protoplasts.

Barley protoplasts were inoculated with BaMV-L RNA alone or co-inoculated with in vitro capped transcripts of sat-

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BaMV variants. Northern (RNA) blot analyses of total RNAs from inoculated protoplasts 24 h post-inoculation (h.p.i.) revealed that the satBaMV transcripts BSL1, BSL2, BSL3, BSL4, BSL6, BSUSA, and BSF4 were all biologically active when co-inoculated with helper BaMV-L RNA (Fig. 1B). However, the accumulation of BaMV genomic RNA as well as that of two subgenomic RNAs, 2.0 and 1.0 kb in size, in the inoculated protoplasts varied with the satBaMV variant used (Fig. 1A). Variants BSL1, BSL2, BSL3, BSL4, BSF4, and BSUSA had no or slight effects on the accumulation of either genomic

Fig. 1. Northern (RNA) blot analyses of the accumulation of bamboo mosaic potyvirus (BaMV) and satBaMV RNAs in barley protoplasts co-inoculated with BaMV-L RNA and satBaMV variants. Protoplasts were inoculated with BaMV-L RNA alone (–) or co-inoculated with in vitro transcripts of BSL1 (L1), BSL2 (L2), BSL3 (L3), BSL4 (L4), BSL6 (L6), BSF4 (F4), and BSUSA (USA). At 24 h post-inoculation, total RNAs extracted from 3×10^4 protoplasts were glyoxylated, electrophoresed in a 1% agarose gel, and transferred to a nylon membrane. Blots were hybridized with (A) 32 P-labeled BaMV-specific and (B) satBaMV-specific probes. Exposure times to X-ray film were (A) 16 and (B) 2 h, representing an approximately eightfold difference. Positions of the BaMV genomic RNA (6.4 kb), 2.0-kb (2.0 kb) and 1.0-kb (1.0 kb) subgenomic RNAs, and satellite RNA (satBaMV) are indicated on left. C, Relative average levels for genomic RNA accumulation obtained from three independent experiments along with standard error bars.

RNA or the two subgenomic RNAs (Fig. 1A and C). However, in protoplasts co-inoculated with BSL6 satBaMV, the accumulation of genomic and subgenomic RNAs was severely affected and the reduction was significant (lane L6). BSL6 satBaMV reduced the amount of BaMV genomic RNA to a level 10 to 15% of those found in protoplasts inoculated with BaMV-L RNA alone. Consistent results were obtained in three independent experiments with different protoplast preparations (Fig. 1C).

Effects of BSL6 satBaMV on local lesion production and genomic RNA accumulation in *Chenopodium quinoa*.

To evaluate whether BSL6 satBaMV has any effects on symptom modulation in plants, leaves of *C. quinoa* were inoculated with helper BaMV-L or BaMV-S RNA alone or co-inoculated with BSF4 and BSL6 satBaMV, respectively. Table 1 shows that BaMV-L or BaMV-S RNA alone induced local lesion production with an average of about 140 lesions per leaf from three independent experiments. The presence of BSF4 satBaMV resulted in an average lesion number of 114 or 215, depending on the helper virus used. BSL6 satBaMV had a considerable adverse effect on local lesion formation, with lesion numbers at a level 12 to 15% of those produced by BaMV-L or BaMV-S RNA alone (Table 1).

To determine the levels of genomic and sat RNA accumulation in *C. quinoa* plants, total RNA or virion RNA extracts were prepared from inoculated leaves at 7 days post-inoculation (d.p.i.). As shown in Figure 2A, the BaMV genomic 3' end-specific probe detected similar levels of accumulation of both the BaMV-S genomic RNA and 1.0-kb subgenomic RNA in the total RNA extracts from leaves inoculated with either BaMV-S RNA alone or co-inoculated with BSF4 transcripts. In contrast, BSL6 greatly suppressed the accumulation of both BaMV-S genomic RNA and 1.0-kb subgenomic RNA in inoculated leaves, to a level 2 to 12% of that induced by BaMV-S RNA alone in three independent experiments (Fig. 2A, lane L6). Similar results were also observed in a Northern blot of virion RNAs, except that the 1.0-kb subgenomic RNA was not detected (Fig. 2B). The latter is believed to be due to inefficient encapsidation of the subgenomic RNA of BaMV-S (N.-S. Lin, *unpublished*). The satBaMV-specific probe detected a large accumulation of BSF4 in leaves co-inoculated with BaMV-S RNA when total RNA was examined (Fig. 2C, lane F4), but BSL6 was detectable at a substantially lower level (lane L6). The level of BSL6 satBaMV detected was between 1 and 8% of that for BSF4 sat-

Table 1. Production of local lesions in *Chenopodium quinoa* co-inoculated with bamboo mosaic virus (BaMV) RNA and satBaMV variants

Inocula ^a	Avg. no. of local lesions/leaf ^b			
	Exp. 1	Exp. 2	Exp. 3	Avg. (%)
BaMV-S RNA only	113 ± 14	238 ± 36	52 ± 6	134 (100.0)
BaMV-S RNA + BSF4	115 ± 28	151 ± 48	78 ± 30	114 (85.6)
BaMV-S RNA + BSL6	17 ± 8	12 ± 2	19 ± 7	16 (11.9)
BaMV-L RNA only	133 ± 19	125 ± 32	171 ± 40	143 (100.0)
BaMV-L RNA + BSF4	251 ± 44	185 ± 28	210 ± 11	215 (150.0)
BaMV-L RNA + BSL6	17 ± 3	9 ± 3	39 ± 21	22 (15.2)

^a For inoculum, 1 µg of BaMV genomic RNA was used alone or mixed with satBaMV transcripts derived from 0.6 µg of plasmid DNA.

^b Each inoculum was inoculated onto four leaves of each plant. Data present the average numbers ± standard errors of lesions per leaf from four inoculated plants.

BaMV in three independent experiments. Similar relative amount of BSF4 and BSL6 were also found in virion RNAs purified from inoculated leaves (Fig. 2D), indicating that both BSF4 and BSL6 satBaMVs were encapsidated. Thus, the low level of BSL6 satBaMV detected in the inoculated leaves should not be residual RNA from the inoculum.

Effects of BSL6 satBaMV on the symptoms in *Nicotiana benthamiana* induced by BaMV-S.

To test for the effects of BSL6 satBaMV on symptom formation in *N. benthamiana*, BaMV-S was used as a helper be-

cause it causes more severe symptoms than BaMV-L; the latter strain only induces a transient mild mottle on this host plant (Chang et al. 1997). In each experiment, 12 plants were inoculated with BaMV-S RNA alone or co-inoculated with either BSF4 or BSL6 transcripts. All plants inoculated only with BaMV-S RNA exhibited severe necrotic symptoms on the inoculated leaves at 7 to 10 d.p.i. Necrotic and mosaic symptoms appeared on the upper noninoculated leaves at 10 to 14 d.p.i. (Fig. 3, left). Similarly, severe symptoms were observed in all 12 plants co-inoculated with BaMV-S and BSF4 (Fig. 3, middle). However, symptoms in plants co-inoculated with BaMV-S and BSL6 were suppressed (Fig. 3, right). Of the 12 plants co-inoculated with BaMV-S and BSL6, one showed a mild mosaic symptom at 12 d.p.i., while three showed mosaic and the remaining were free from symptoms at 24 d.p.i. Similar results were obtained in six independent inoculation experiments.

Effects of BSL6 satBaMV on the accumulation of genomic RNA and capsid protein in the infected *N. benthamiana*.

Northern blot analyses of total RNAs from inoculated leaves of *N. benthamiana* 12 d.p.i. showed results similar to those obtained from inoculated *C. quinoa*; i.e., BSL6 satBaMV greatly reduced the accumulation of genomic and subgenomic RNAs, to a barely detectable level. BSL6 satBaMV only accumulated to 1 to 5% of the level of BSF4 (Fig. 4A). The encapsidated BSF4 and BSL6 satBaMVs also were found in virion RNAs purified from the inoculated leaves of *N. benthamiana* 12 d.p.i. (data not shown).

For assays of upper noninoculated leaves co-infected with BaMV-S and BSL6, symptomatic and asymptomatic leaves were collected separately. By 12 and 24 d.p.i., all plants inoculated with BaMV-S RNA only or co-inoculated with BSF4 showed severe symptoms. They accumulated a high level of genomic and subgenomic RNAs of BaMV as well as a high level of BSF4 satBaMV in the noninoculated symptomatic leaves (Fig. 4B and C). In contrast, the accumulation of genomic RNA was severely depressed in the upper asymptomatic leaves in plants co-inoculated with BSL6 at these two time periods (Fig. 4B and C). However, plants co-inoculated

Fig. 2. Accumulation of bamboo mosaic potexvirus (BaMV) and sat-BaMV in inoculated *Chenopodium quinoa* leaves 7 days post-inoculation. Total RNA (A, C) extracted from 10 mg of leaves or virion RNA (B, D) extracted from 100 mg of leaves inoculated with BaMV-S RNA alone (–) or together with either BSF4 (F4) or BSL6 (L6) transcripts were subjected to RNA gel blot analysis as described in Figure 1. Blots were hybridized with (A, B) BaMV-specific or (C, D) satBaMV-specific probes, respectively. H is the mock-inoculated control.

Fig. 3. Leaves of systemically infected *Nicotiana benthamiana* plants. Plants were inoculated with BaMV-S RNA alone (left) or co-inoculated with transcripts of either BSF4 (middle) or BSL6 (right). Photograph was taken of upper, noninoculated leaves 2 weeks after infection.

Fig. 4. Accumulation of bamboo mosaic potexvirus (BaMV) RNA, satBaMV, and capsid protein in infected *Nicotiana benthamiana*. Plants were inoculated with BaMV-S RNA alone (–) or together with either BSF4 (F4) or BSL6 (L6) transcripts. H is the mock-inoculated control. Inoculated (A, D) and upper noninoculated (B, C) leaves were harvested at 12 (A, B, D) and 24 (C) days post-inoculation for Northern (RNA) blot or Western blot (immunoblot) analysis. A–C, Northern blot analyses of BaMV and satBaMV RNA accumulation. Asymptomatic (As) and symptomatic (S), upper noninoculated leaves inoculated with a mixture of BaMV-S and BSL6 RNAs were separately processed. Northern analyses of total RNA from 10 mg of leaves were as described in Figure 1. D, Western blot analysis showing capsid protein (CP) accumulation by anti-BaMV capsid protein serum followed by reaction with goat anti-rabbit IgG conjugated horseradish peroxidase and detected on the ECL Western blotting system (Amersham, Buckinghamshire, England).

with BaMV-S and BSL6 and showing mosaic symptoms accumulated a level of genomic and subgenomic RNAs in the upper symptomatic leaves similar to those in plants inoculated with BaMV-S RNA alone (Fig. 4C). BSL6 RNA was also not detectable in the upper noninoculated leaves, whether symptomatic or asymptomatic, at 12 and 24 d.p.i. (Fig. 4B and C).

Inoculated leaves of *N. benthamiana* described as above were also analyzed at 12 d.p.i. for capsid protein production by Western blot (immunoblot) with anti-BaMV-capsid protein serum. As shown in Figure 4D, a high level of capsid protein was detected in the inoculation with BaMV-S alone, or together with BSF4 satBaMV, and no nonspecific bands were detected in the mock-inoculated leaves. However, there was a low amount of capsid protein detected in leaves co-inoculated with BSL6. These results are consistent with the low production of 1.0-kb subgenomic RNA in the BSL6 co-inoculated leaves (Fig. 4A).

Replication of BSF4 and BSL6 in the inoculated protoplasts of *N. benthamiana*.

Although both BSF4 and BSL6 satBaMVs replicated well in the co-inoculated barley protoplasts (Fig. 1B), BSL6 did not accumulate to a level comparable to that of BSF4 in inoculated leaves of *C. quinoa* and *N. benthamiana* (Figs. 2C and D, 4A). Experiments were conducted with protoplasts of *N. benthamiana* to evaluate their ability to support the satBaMVs replication at the single-cell level with BaMV-L or BaMV-S RNA as a helper. As shown in Figure 5A, both BSF4 and BSL6 replicated to comparable levels in the protoplasts at 24 h.p.i., as determined by Northern blot analysis with a satBaMV-specific probe, when either the BaMV-L or BaMV-S RNA was used as a helper. The accumulation of both BaMV-L and BaMV-S genomic and subgenomic RNAs substantially

Fig. 5. Northern (RNA) analyses of (+)-strand and (–)-strand RNA accumulation of bamboo mosaic potexvirus (BaMV) and satBaMV in infected protoplasts of *Nicotiana benthamiana* 24 h post-inoculation. Total RNAs were extracted from 3×10^4 protoplasts inoculated with helper BaMV-L or BaMV-S RNA alone (–) or together with either BSF4 (F4) or BSL6 (L6) transcripts. Accumulation of (A) (+)-strand or (B) (–)-strand RNA of BaMV and satBaMV was detected with a BaMV or satBaMV-specific probe (see Materials and Methods). Exposure times to X-ray film for detection of (B) (–)-strand RNA were approximately 10-fold longer than those for (A) (+)-strand RNA.

decreased in the protoplasts co-inoculated with BSL6 satBaMV (Fig. 5A). This indicates a similar ability of the two satBaMVs to replicate in the protoplasts of *N. benthamiana* when supported by two BaMV isolates tested.

To analyze the accumulation of (–)-strand RNA of BaMV and satBaMV, the blots of total RNAs from inoculated protoplasts of *N. benthamiana* at 24 h.p.i. were probed with (–)-strand specific probes. As shown in Figure 5B, the minus-strand BaMV RNA accumulated to similar levels in samples with or without the satBaMV co-inoculation. The addition of BSL6 in the inoculum did not affect the (–)-strand accumulation (Fig. 5B, lane L6), although it greatly reduced the (+)-strand level of BaMV RNA (Fig. 5A). The (–)-strand of BSF4 and BSL6 was detected at a level similar to their (+)-strand accumulation (Fig. 5B).

DISCUSSION

We have demonstrated that different satBaMV variants (Liu et al. 1997) had different abilities to interfere with BaMV replication. Among them, BSL6 satBaMV is unique since it substantially decreased the accumulation of its helper virus and caused an attenuation of symptoms. In protoplasts, the presence of BSL6 satBaMV resulted in an 85 to 90% reduction of the viral genomic RNA at 24 h.p.i., whereas the BSL6 was consistently amplified to high levels by the helper BaMV (Figs. 1 and 5). A reduced accumulation of the viral genomic RNA was also observed in *C. quinoa* (Fig. 2) and *N. benthamiana* (Fig. 4) co-inoculated with BSL6, although the BSL6 satBaMV could be detected at a very low level. BSL6-mediated interference occurred at the genomic RNA, subgenomic RNA, and capsid protein levels; however, the synthesis of (–)-strand was virtually unaffected both in co-inoculated protoplasts (Fig. 5B) and plants (data not shown). It has been shown that 5' untranslated regions (UTRs) of potexviruses contain multiple *cis*-acting elements that are required for the synthesis of both genomic and subgenomic RNAs, but not for (–)-strand RNA synthesis (Kim and Hemmenway 1996). BaMV and satBaMV variants share 55 to 63% identity at the nucleotide level in their 5' UTRs (Lin et al. 1997). It is likely that the interactions between the regulatory signals in the BaMV 5' UTR with the RNA-dependent RNA polymerase and/or host factors were interfered with by BSL6 satBaMV. This subsequently resulted in the preferential reduction of the (+)-strand RNA accumulation. Moreover, the accumulation of capsid protein was noticeably reduced in the presence of BSL6 satBaMV. Thus, the reduction of BaMV genomic RNA affected by BSL6 satBaMV may be a result of the interference of (+)-strand viral RNA synthesis rather than by encapsidation.

BSL6 satBaMV can attenuate the symptoms produced by the helper BaMV-S and BaMV-L in the inoculated *C. quinoa* (Table 1) and *N. benthamiana* (Fig. 3). The symptom attenuation was correlated with a reduction in viral RNA and capsid protein levels in inoculated and noninoculated leaves, but was independent of the level of BSL6 satBaMV molecules in plants (Figs. 2 and 4). BSL6 satBaMV differs at 7% of its nucleotide sequence from BSF4, a non-attenuating satBaMV (Liu et al. 1997). The sequence differences in satBaMV variants may result in different symptom modulations, which may involve the interactions of satBaMV,

helper virus, and host plants. The mRNA-type sat RNA of ArMV-mediated symptom amelioration is host specific, and symptom attenuation was not correlated with the concentration of helper virus (Liu et al. 1991). The concomitant reduction of helper virus RNA levels and symptom alleviation have been reported for many small sat RNAs that do not encode any functional ORFs (Collmer and Howell 1992). A single nucleotide or region change in sat RNA sequence may alter the sat RNA-mediated host response or control the regulation of helper virus (Sleat and Palukaitis 1990; Roossinck et al. 1992; Zhang et al. 1994; Taliansky and Robinson 1997).

BSL6 accumulated at significantly lower levels than BSF4 in the BaMV-inoculated leaves of *N. benthamiana* and *C. quinoa* (Figs. 2 and 4). The undetectable level of BSL6 satBaMV in the upper noninoculated leaves may be due to the great reduction of BaMV accumulation by the BSL6 satBaMV. When BaMV accumulation went down to a threshold in the inoculated leaves, the movement of BaMV was affected and, consequently, BSL6 systemic movement assisted by the BaMV helper was affected. The diminished level of BSL6 satBaMV in the leaves might be a result of very low levels of BaMV (+)-strand RNA. Nevertheless, results from protoplast assays (Figs. 1 and 5) demonstrated that two strains of BaMV, BaMV-S and BaMV-L, served as effective helpers to support the replication of BSL6 and BSF4 satBaMVs at the single-cell level, as well as to support the encapsidation of both BSL6 and BSF4 (Fig. 2D). Thus, the low accumulation of BSL6 in the inoculated leaves could be due to inefficiency in spreading in plants rather than the inefficiency of satBaMV replication or encapsidation. Such defects by sat RNA in long-distance movement have been previously noted for Ix-sat RNA associated with cucumber mosaic virus when tomato aspermy virus acts as a helper virus (Moriones et al. 1992, 1994; Bernal and Garcia-Arenal 1994). How BSL6 satBaMV interfered with genomic RNA accumulation and attenuated the virus-induced symptoms in the upper noninoculated leaves remains to be determined.

Taken together, the data suggest that BSL6 satBaMV exhibits many unusual characteristics of mRNA-type sat RNAs. BSL6 satBaMV is able to interfere with genomic RNA replication and attenuate the virus symptoms while it may itself be inefficient for movement. Thus, BSF4 and BSL6 satBaMVs provide excellent materials to study the molecular basis of the mRNA-type sat RNA-mediated reduction of helper virus replication and symptom attenuation, and the systemic movement of the sat RNA itself.

MATERIALS AND METHODS

Virus and satBaMV isolates.

BaMV-L is a satellite-free isolate derived from BaMV-V, as described previously (Lin et al. 1996). BaMV-S is a nitrous acid-induced mutant (Chang et al. 1997) derived from BaMV-O (Lin and Chen 1991), which causes necrotic and mosaic symptoms in *N. benthamiana*. Full-length cDNA clones of satBaMV variants used in this study were the following: pBSL1, originally isolated from *Dendrocalamus latiflorus* 'Subconvex' Lin; pBSL2 and pBSUSA from *Bambusa beecheyana* Munro; pBSL3 from *B. dolichoclade* 'Stripe' Lin; pBSL4 from *Pseudosasa usawai* Makino & Nemoto; pBSL6

from *D. latiflorus* Munro (Liu et al. 1997); and pBSF4 from *B. vulgaris* Schrader ex Wendland (Lin et al. 1996).

Construction of infectious cDNA clones of satBaMV variants.

With the exception of infectious clone pBSF4, the preparation of which was previously described (Lin et al. 1996), the infectious cDNA clones for other satBaMV variants were prepared with primers BS19, 5'-TGCCTGCAGTAATACGAC TCACTATAGAAAAC TCACCGCAACGA-3' (*Pst*I site in boldface, T7 promoter sequence in italic, and 5' terminal 18 nucleotides of satBaMV (Lin and Hsu 1994)) and BS24, 5'-CCTTCTCGAG(T)₁₅-3' (*Xho*I site in boldface) to amplify the cDNA fragments by polymerase chain reaction. The amplified full-length sat cDNAs were then digested with *Pst*I and *Xho*I, and cloned into pUC119 (Sambrook et al. 1989). In vitro transcription was performed as described (Lin et al. 1996).

Protoplast inoculation and RNA isolation.

Protoplasts of barley (*Hordeum vulgare* L. 'Larker') were prepared from 7-day-old seedlings (Lin et al. 1992a) and those of *N. benthamiana* from 1-month-old plants (Weiland and Dreher 1989). For each inoculation, 2 µg of capped satBaMV transcripts was co-inoculated into protoplasts with 1.0 µg of either BaMV-L or BaMV-S viral RNA. The procedures for the protoplast inoculation and RNA extraction were carried out as described previously (Lin et al. 1992a).

Plant inoculation and RNA extraction.

For plant inoculations, 1-month-old plants of *N. benthamiana* and *C. quinoa* were used. RNA transcripts generated from 0.6 µg of plasmid cDNA were mixed with 1 µg of BaMV RNA and co-inoculated onto 3 to 4 leaves of each plant. Leaves were harvested at 7 d.p.i. for *C. quinoa* and 12 and 24 d.p.i. for *N. benthamiana*. Purification of virions and extraction of virion RNA were as described previously (Lin and Chen 1991). Extraction of total RNAs from plants followed the method described by Verwoerd et al. (1989).

Northern and Western blot analyses.

Virion RNAs or total nucleic acids, extracted from protoplasts or from leaves of *C. quinoa* or *N. benthamiana*, were denatured in the presence of glyoxal and then transferred to nylon membrane for Northern blot analysis (Lin et al. 1992b). The ³²P-labeled RNA probe was prepared by linearization of pBaHB with *Hind*III followed by transcription with SP6 RNA polymerase, which is complementary to the 3' ends of (+)-strand BaMV RNA (Lin et al. 1993). Similarly, *Bam*HI restriction and T7 transcription yielded a probe for the detection of (-)-strand BaMV RNA. The probe, specific for the detection of (+)-strand satBaMV, was transcribed from *Eco*RI-linearized pBSHE by T7 RNA polymerase (Lin et al. 1996). Likewise, the probe for the detection of (-)-strand satBaMV was transcribed from *Pst*I-linearized pBSHE by SP6 RNA polymerase.

For Western blot analysis, a blot of total proteins extracted from inoculated leaves of *N. benthamiana* and separated in 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis was probed with anti-BaMV-capsid protein serum (Lin and Chen 1991), followed by the detection of an ECL Western Blotting system (Amersham, Buckinghamshire, England).

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