Material and Methods

Construction of virus-based vectors

A blunt-ended cDNA of the intron (IV2) derived from the potato (*S. tuberosum*) ST-LS1 gene (38, AN X04401) was generated de novo by sequential nested PCR and modified to introduce a *Kpn*I site between positions 94 and 95. The coding region of the GFP was then interrupted by insertion of the intron (IV2) between positions 174 and 175. This construct was sequenced and identified as IV2/GFP. The IV2/GFP construct was digested with *Kpn*I to clone the monomeric (plus strand) full-length ELVd (from nt 1 to 333), generated by PCR from the cDNA of ELVd (AN-AJ536613) (Fadda et al., 2003). The selected positions of the 5' and 3' ends of the full-length ELVd cDNA mimic the product of the hammerhead-mediated cleavage and consequently prevent spontaneous cleavage of the (+) ELVd-RNA in the chimeric transcripts used in this study. The resulting vector identified as ELVd(+)IV2/GFP contained the plus strand of the monomeric ELVd embedded in the IV2/GFP construct (Supporting Figure 4) . The GFP, IV2/GFP and ELVd(+)/IV2/GFP constructs were subsequently cloned into the *Cla*I site of the PVX-based vector pP2C2S (Chapman et al., 1992), to generate the plasmids PVX-GFP, PVX-GFP/IV2 and PVX- GFP/IV2/ELVd(+).

Plant inoculation

Infectious PVX transcripts were synthesized *in vitro* with T7 RNA Polymerase (Takara), according to the manufacturer's instructions, using the PVX-GFP, PVX-IV2/GFP and PVX-ELVd(+)/IV2/GFP plasmids linearized with *Spe*I as DNA templates. After capping using the ScriptCap m7G Capping System (Epicentre® Biotechnologies, Madison, WI, USA) according to the manufacturer's instructions, the transcripts were used to inoculate *N. benthaminana* plants as previously described (Zhao et al., 2001).

Analysis of GFP expression

GFP expression was analyzed under a Leica MZ 16 F fluorescence stereomicroscope equipped with filters DSR, GFP2 and V (Leica). Tissue sections were also observed under a TCS SL confocal laser scanning microscope (Leica), with excitation at 488 nm and emission at 510–560 nm. GFP was also detected by Western blot using GFP-specific antibodies, as previously described (Gomez and Pallas, 2007).

RT-PCR analysis

Total RNA was extracted from leaves of the PVX-infected and mock-inoculated *N. benthamiana* plants at 10 and 25 days post-inoculation using TRI reagent (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. RT-PCR analysis was performed using the SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase [Invitrogen Corporation, Carlsbad, CA, USA] according to the manufacturer's instructions (DMSO [2%] was added to the RT-PCR mix to facilitate the amplification of the highly structured intron sequence). The relative sequence position of the specific primers GFP-1/GFP-2 and IV2-1/IV2-2 used to amplify the full-length GFP mRNA and IV2 intron is shown in Supporting Figure 2. The primers PVX-1 (5´CACACTTAGGCAATTTTGCATGAAG) and PVX-2 (5´GTGGTAGAGTGACAACAGCCTCAGC) flanking a region (~350 nt) of coat protein mRNA of the PVX, were used to confirm systemic infection by RT-PCR.

Plasmid construction

The full-length sequence (plus strand) of the Eggplant latent viroid was obtained by PCR, starting from the cDNA of ELVd (AN-AJ536613) (Fadda et al., 2003). The amplified monomeric cDNA (from nt 1 to 333), was cloned in a binary plasmid pMOG 800 carrying a modified GFP cDNA (with a *Kpn*I site at the 5´end) under the control of the Cauliflower mosaic virus 35S promoter and the *nopaline synthase* terminator (t-Nos). The resulting vector ELVd-5'UTR/GFP contained an ELVd cDNA fused as a 5' untranslated region (UTR) to the GFP cDNA (Supporting Figure 4).

Agro-infiltration

N. benthamiana plants were infiltrated with the ELVd-5'UTR/GFP or unmodified GFP constructs as previously described (Gomez and Pallas, 2007) and maintained at 28 °C, with 14 h light. GFP expression in plants was analyzed at 72 h after agro-infiltration with a TCS SL confocal laser scanning microscope (Leica), with excitation at 488 nm and emission at 510–560 nm. The chloroplastic localization of the ELVd-5'UTR/GFP was established using, as a reference, a GFP fused to a chloroplast-specific transit peptide derived from a protein of the Oxygen evolving complex (OE23) (Roffey and Theg, 1996)]. The GFP was also detected by Western blot using GFP-specific antibodies, as previously described (Gomez and Pallas, 2007).