Supplemental TABLE S1. Deoxyribonucleotide primers used for RT-PCR amplification of the different eggplant tRNA ligase cDNAs

Primer [a]	Sequence (5' to 3')[b]	Description
I	CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTT TTTTTTTTTTTTTVN	RT poly(A) tail
II	ATCACCTTTATGATGGAAAGAGCCG	PCR first cDNA
III	GCCGCGCCCTCGAGAAAGAATTCGAGAGTGCCAG	
IV	CCAGTGAGCAGAGTGACG	3' RACE (nested), also with primers I and II
V	AGAGTGAACTTATTGAACGTTTTGG	
VI	GAGGACTCGAGCTCAAGC	
VII	CCGGTGGATTTATATCAATGCGG	First 5' circular RACE (nested)
VIII	GTTGCCGCAAAAGATGCCAATACAC	
IX	GTCTGTACAGACTGAGTCCCTCC	
X	CCAATACACTACCACAGTTACTTTCC	
XI	CCCTCCTCGATAATAGACCTCACAG	
XII	GGCGCGGCGCTAGCATGCCAAACAATCAGAGAAAG	RT-PCR cDNA 5' region (hemi-nested), also with primers VII and XII
XIII	TCCTGGAAAAAAAACGATGAGACCC	
XIV	TGGAAATATTAGGGCTTGAAGGAGC	
XV	GGTGCTAATCCAACGCTGCCAAAC	Second 5' circular RACE (nested) for the genuine 5' end
XVI	TATTACTGAAAGTGGTGCACAGTC	
XVII	GGCCTCACTTGCCATTTTTTTCCTTC	
XVIII	GTGGTGCACAGTCTAGTGTTCCAG	
XIX	CCACCCCTTTCTCTGATTGTTTGG	
XX	CACTCAGAACCCATGTAGGGTTCAC	RT-PCR (nested) full-length open reading frame
XXI	CCTTTTGTGTAGAACTGAATGTCGG	
XXII	GAGGAATTACAAGTAGAGTTCAAAAG	
XXIII	GGCGCGGCCCATGGATGTCGGTTTCGCATAG	
XXIV	GGCGCGGCGCGGCCGCAAAGAATTCGAGAGTG	
XXV	ATGTCGGTTTCGCATAGGGTC	Deletion N-terminal extra amino acids of recombinant tRNA ligase
XXVI	GGTATATCTCCTTCTTAAAG	
XXVII	CACCACCACCACCACCACTGAGATCCGGCTG	Deletion C-terminal extra amino acids of recombinant tRNA ligase
XXVIII	AAAGAATTCGAGAGTGCCAG	

[a]Positions of primers I to III, V, VII to XXV and XXVIII in eggplant tRNA ligase cDNA sequence are shown in Supplementary Fig. 1. Primers IV, VI, XXVI and XXVII do not correspond to tRNA ligase cDNA. Primers IV and VI are homologous to the 5' sequence extension of primer I. Primers XXVI and XXVII are homologous to different regions of plasmid pET23d(+).

[b]Primers XII, XIII, XIV and XIX contain some point mutations respect to the eggplant tRNA ligase cDNA sequence finally determined.

Supplemental FIG. 1. Nucleotide sequence of the cDNA corresponding to eggplant tRNA ligase mRNA (JX025157). The 5' and 3' untranslated regions are in blue, the coding region in black, the sequence corresponding to the predicted (ChloroP) transit peptide on green background and the fragment used for virus-induced gene silencing of *N. benthamiana* tRNA ligase underlined. The positions of primers I to III, V, VII to XXV and XXVIII used in the different RT-PCR amplifications are indicated by arrows under the sequence. Asterisks in some of the primers indicate 5' extensions not corresponding to the tRNA ligase sequence.

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GTGACTGGCTCAAAGGCGGTAGTAGTGGCTCTTCTTCCCCGCTGGAATTTCTCGCCTATTTTATATTGATTGACAGCAGCCGAGTCTTCCTTTTGTGTAGAACTGAATGTCGGTTTCGCATAGGGTCATT
                                                                                                                /----------------------> XXI
                                                                                                                   *---------------> XXIII
                                                                                                          XXV /------------------->

TACTCTTTCACTCATTACAAACTCTATAATCTCTCTTCTTCTTTATCATCTTTGCCTTCTAGAATCTTCTTCCCTTTTCAATCTCCTTCCTTTCACACGTTCTCTTCACTCATGCCCAACAATCAGGAAA
                                                                                                                XII *-------------------
                                                                                                                     XIX <--------------

GGGGTGGTTATGAAGGAAAAAAATGGCAAGTGAGGCCAAGTTCCAATAGGGTACCAGGCTCGTCTTCAAATGTGGAACCTGTATCTGCTGCAACTGCTGAAGCCATTACCGACCGTCTAAAGTCCGTGGA
>     XVII <------------------------/
------/

TATTACTGAAAGTGGTGCACAGTCTAGTGTTCCAGTCACATCTCTTCAGTTTGGCAGCGTTGGATTAGCACCCCAGTCACCTGTGCAACATCAAAAAGTAATCTGGAAACCCAAATCATATGGAACAGTG
/----------------------> XVI                 XV <----------------------/
          /----------------------> XVIII

TCTGGAGCCCCAGTGGTTGAAGCTGGAAAAACACCAGTTGAACAAAAAAGTGCTCTTTTAAGTAAATTATTCAAGGGTAATTTATTGGAAAATTTTACTGTAGATAACTCAACATTCTCGAGAGCCCAAG
TAAGGGCCACTTTCTACCCAAAATTTGAGAATGAGAAATCAGATCAGGAGATCAGGACAAGGATGATAGAGATGGTCTCCAAAGGCTTGGCTATAGTCGAGGTCACACTTAAGCATTCTGGATCTCTTTT
TATGTATGCTGGGCATGAAGGTGGAGCATATGCCAAGAATAGCTTCGGGAATATCTATACTGCCGTTGGCGTCTTTGTTCTTGGACGGATGTTTCGTGAGGCATGGGGAACTAAAGCAAGCAAGAAGCAA
GCAGAGTTCAATGAGTTTCTTGAGCGCAATCGTATGTGCATATCAATGGAGTTGGTCACGGCAGTGTTGGGGGACCACGGACAACGCCCACGAGATGATTATGCGGTTGTGACTGCAGTCACGGAGTTGG
GAAATGGAAAACCAACTTTCTATTCAACTCCCGATGTAATTGCTTTTTGCAGGGAATGGCGATTACCAACAAATCATGTATGGCTGTTCTCAACAAGGAAATCAGTGACTTCCTTCTTTGCTGCGTATGA
TGCACTTTGCGAGGAAGGTACAGCAACCACCGTTTGCGAGGCTCTCAGCGAAGTTGCTGATATTTCTGTACCTGGATCAAAAGACCATATAAAAGTGCAGGGTGAAATTTTGGAGGGTCTCGTGGCCCGC
ATCGTAAAACGTGAGAGCTCAGAGCATATGGAGCGGGTTCTGAGAGATTTTCCTCCTCCGCCATCAGAGGGTGAGGGTTTGGACCTGGGACCTACGTTACGTGAAATTTGTGCTGCAAACAGATCAGAAA
AGCAGCAAATAAAGGCACTTCTTCAGAGTGCTGGCACGGCTTTCTGCCCGAATTATTTGGACTGGTTTGGAGATGAAAACTCTGGTTCACATTCAAGAAATGCTGATCGATCTGTTGTCTCAAAGTTCTT
ACAATCACATCCTGCTGATCTTTATACAGGAAAAATACAGGAAATGGTTCGCTTGATGAGGGAAAAGCGCTTTCCTGCTGCTTTCAAGTGTCATTATAACTTACATAAAATTAATGATGTATCGAGTAAC
AACCTGCCTTTCAAAATGGTGATCCATGTATATAGTGATTCAGGCTTCCGCCGGTACCAGAAAGAGATGAGGCACAAACCAGGACTATGGCCTTTGTATCGAGGCTTTTTTGTTGACCTGGATTTATTCA
AGGTCAATGAGAAGAAAACTGCTGAAATGGCAGGAAGCAACAATCAAATGGTAAAAAATGTGGAAGAGGACAACAGTTTAGCTGATGAAGATGCAAATCTGATGGTCAAGATGAAATTTCTTACTTACAA
GTTGAGAACTTTTTTGATCCGTAATGGCTTGTCGACTCTTTTCAAAGAAGGACCTTCTGCGTATAAGTCTTATTACCTGAGGCAAATGAAAATTTGGAATACTTCAGCAGCCAAGCAACGAGAACTCAGC
AAGATGCTTGATGAATGGGCAGTATATATACGCAGAAAATATGGGAACAAACCATTGTCATCATCCACATACCTAAGTGAAGCTGAGCCTTTCCTTGAACAATATGCAAAGCGTAGTCCACAAAATCATG
CTTTGATAGGATCTGCTGGAAATTTTGTCAAAGTTGAAGATTTCATGGCTATTGTTGAAGGAGAAGATGAAGAGGGTGATCTCGAGCCTGCGAAAGATATTGCTCCTTCAAGCCCTAGTATTTCCACCAG
                                                                                                                XIV <----------------------/
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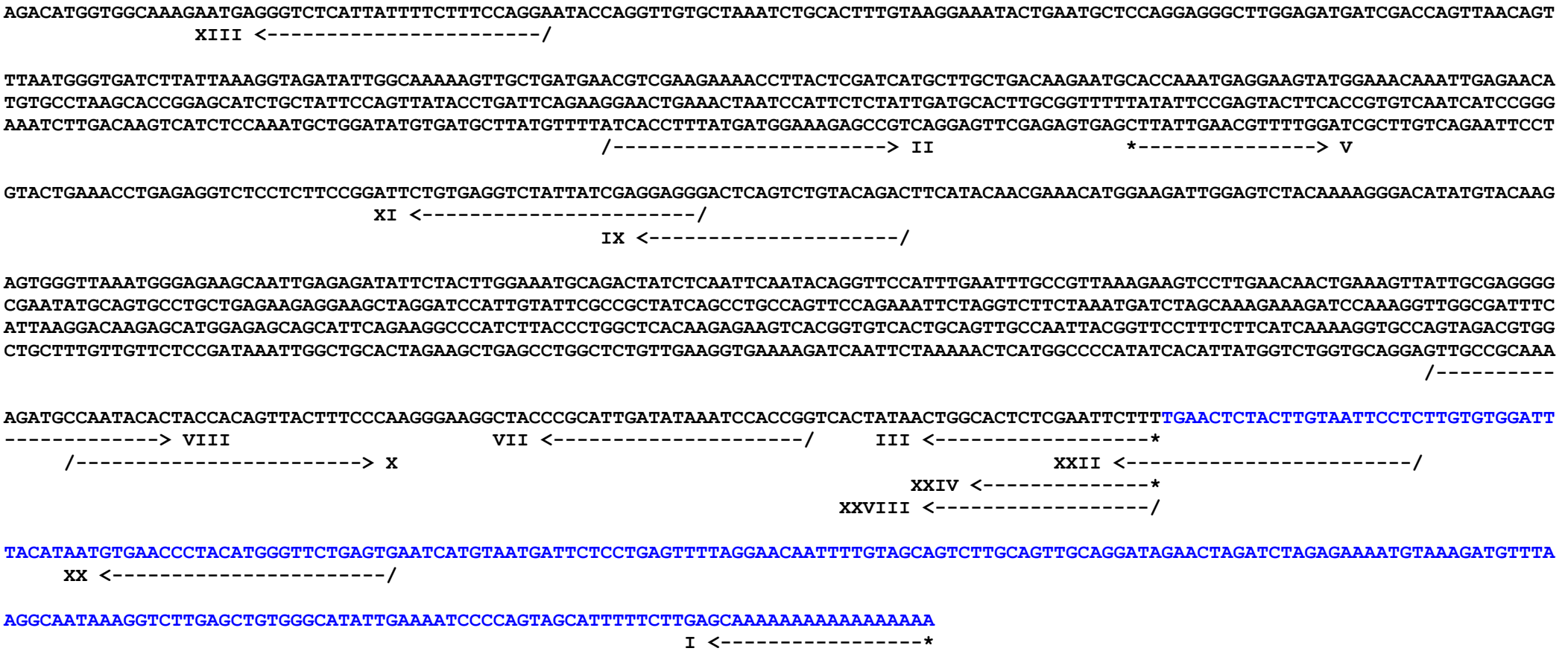
AGACATGGTGGCAAAGAATGAGGGTCTCATTATTTTCTTTCCAGGAATACCAGGTTGTGCTAAATCTGCACTTTGTAAGGAAATACTGAATGCTCCAGGAGGGCTTGGAGATGATCGACCAGTTAACAGT
XIII
TTAATGGGTGATCTTATTAAAGGTAGATATTGGCAAAAAGTTGCTGATGAACGTCGAAGAAAACCTTACTCGATCATGCTTGCTGACAAGAATGCACCAAATGAGGAAGTATGGAAACAAATTGAGAACA
TGTGCCTAAGCACCGGAGCATCTGCTATTCCAGTTATACCTGATTCAGAAGGAACTGAAACTAATCCATTCTCTATTGATGCACTTGCGGTTTTTATATTCCGAGTACTTCACCGTGTCAATCATCCGGG
AAATCTTGACAAGTCATCTCCAAATGCTGGATATGTGATGCTTATGTTTTATCACCTTTATGATGGAAAGAGCCGTCAGGAGTTCGAGAGTGAGCTTATTGAACGTTTTGGATCGCTTGTCAGAATTCCT
II
V
GTACTGAAACCTGAGAGGTCTCCTCTTCCGGATTCTGTGAGGTCTATTATCGAGGAGGGACTCAGTCTGTACAGACTTCATACAACGAAACATGGAAGATTGGAGTCTACAAAAGGGACATATGTACAAG
XI
IX
AGTGGGTTAAATGGGAGAAGCAATTGAGAGATATTCTACTTGGAAATGCAGACTATCTCAATTCAATACAGGTTCCATTTGAATTTGCCGTTAAAGAAGTCCTTGAACAACTGAAAGTTATTGCGAGGGG
CGAATATGCAGTGCCTGCTGAGAAGAGGAAGCTAGGATCCATTGTATTCGCCGCTATCAGCCTGCCAGTTCCAGAAATTCTAGGTCTTCTAAATGATCTAGCAAAGAAAGATCCAAAGGTTGGCGATTTC
ATTAAGGACAAGAGCATGGAGAGCAGCATTCAGAAGGCCCATCTTACCCTGGCTCACAAGAGAAGTCACGGTGTCACTGCAGTTGCCAATTACGGTTCCTTTCTTCATCAAAAGGTGCCAGTAGACGTGG
CTGCTTTGTTGTTCTCCGATAAATTGGCTGCACTAGAAGCTGAGCCTGGCTCTGTTGAAGGTGAAAAGATCAATTCTAAAAACTCATGGCCCCATATCACATTATGGTCTGGTGCAGGAGTTGCCGCAAA
AGATGCCAATACACTACCACAGTTACTTTCCCAAGGGAAGGCTACCCGCATTGATATAAATCCACCGGTCACTATAACTGGCACTCTCGAATTCTTTTGAACTCTACTTGTAATTCCTCTTGTGTGGATT
VIII
VII
III
X
XXII
XXIV
XXVIII
TACATAATGTGAACCCTACATGGGTTCTGAGTGAATCATGTAATGATTCTCCTGAGTTTTAGGAACAATTTTGTAGCAGTCTTGCAGTTGCAGGATAGAACTAGATCTAGAGAAAATGTAAAGATGTTTA
XX
AGGCAATAAAGGTCTTGAGCTGTGGGCATATTGAAAATCCCCAGTAGCATTTTTCTTGAGCAAAAAAAAAAAAAAAA
I

Supplemental FIG. 2. Effect of tRNA ligase silencing on circularization of ELVd and CCCVd monomeric RNAs *in planta*. Dimeric (+) ELVd (A and B) and CCCVd transcripts (C and D) were infiltrated in *N. benthamiana* 16c plants pre-inoculated with a VIGS vector to silence the GFP transgene (A and C) or the endogenous tRNA ligase (B and D). RNAs were purified at 2 (lanes 1 to 3), 3 (lanes 4 to 6), 4 (lanes 7 to 9), 5 (lanes 10 to 12) and 10 (lanes 13 to 15) days from the agroinfiltrated areas of three different plants infected with each VIGS vector. Monomeric circular and linear (+) ELVd (A and B) and CCCVd RNAs (C and D) were revealed by northern blot hybridization. The positions of monomeric circular (mc) and linear (ml) forms of ELVd and CCCVd are indicated on the left of panels. As a loading control, each panel includes a fragment of the corresponding polyacrylamide gel stained with ethidium bromide showing 5S rRNA.