

A Novel Self-Cleaving Viroid-Like RNA Identified in RNA Preparations from a Citrus Tree Is Not Directly Associated with the Plant

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Supplementary material

Table S1. Primers used in this study

Figure S1. Pattern syntax used to search for hammerhead ribozyme structure in citrus RNA seq library

Figure S2. Contig containing the sequences conserved in most natural hammerhead ribozymes in each polarity strand and a terminal direct repeat.

Figure S3. CtaHVd-LR1 (+) or (–) transcript RNAs detected by Northern-blot hybridization with equalized full-length digoxigenin-labeled riboprobes.

Figure S4. Multiple-sequence alignment of full-length cDNAs of hammerhead viroid-like RNA 1 variants.

Figure S5. PCR and RT-PCR assays using DNA and RNA preparations from isolate 14A, respectively.

Table S1. Primers used in this study

Name	Sequence (5' to 3')	position*	Used for
CH1 Rev	GTAAGAGCAGAGCCCTCATTTCTA	140-117	PCR of full-length CtaHVd-LR1 cDNA (coupled with CH2 For) and 5' RACE of the 3' fragment generated by the (+) HHRz self-cleavage
CH2 For	AGTATAATGGCGAGGTACCGTG	141-163	PCR of full-length CtaHVd-LR1 cDNA (coupled with CH1 Rev) and 5' RACE of the 3' fragment generated by the (-) HHRz self-cleavage
CH3 Rev	CCTGGTCTCATCAGGCGC	34-17	PCR of full-length CtaHVd-LR1 cDNA (coupled with CH4 For)
CH4 For	CAGGTCGAAACACCTATTTGCT	35-56	PCR of full-length CtaHVd-LR1 cDNA (coupled with CH3 Rev)
CH5 Rev	AAACCGACCTCCGCAACTTCTG	444-423	PCR of full-length CtaHVd-LR1 cDNA (coupled with CH6 For)
CH6 For	GGGATCCAACCTAGCGGTTAAAT	445-468	PCR of full-length CtaHVd-LR1 cDNA (coupled with CH5 Rev)

* referred to variant CtaHVd-LR1_3

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* COMMAND
=====
// COMMAND Hammer Head
=====

//-----
// Hammer Head
//-----

R110 = {AT, TA, GC, CG, TG, GT}
L110 = 3...5 // Stem I
L120 = RTM
L130 = 3...7 // Stem II
L131 = 3...15 // loop II
L140 = CTGANGA
L141 = 0...2
L150 = G
L160 = 3...20 // Stem III
L170 = 3...50 // loop III
L180 = C
L190 = GAAAY

=====
// PATTERN SYNTAX
=====

//-----
// Hammer Head
//-----

P1 = L110 L120 P2 = L130 L131 R110~P2 L141 L140 L141 L150 P3 = L160 L170 R110~P3 L180 L141 L190 R110~P1

```

Figure S1. Pattern syntax used to search for hammerhead ribozyme structure in citrus RNA seq library

>NODE_11827_length_651_cov_57.163636

Monomer (550 nt) { TTGAAGGAAGAAGTTG**TGGGTGTAGTGCCCTGTCGCCGGGGCGCCTGATGAGACCAAGGCA**
GGTCGAAACACCTATTTGCTGGCGAGGTCCGGGAACCTTGTTATGTCAAAAGAGAGGGTA
AATCGGGGAAACTCGCCTCCTAGAAATGAGGGCTCTGCTCTTACAGTATAATGGCGAGGT
ACCGTGCAGTTAGCAAGGGATTGCTAGCTTCTTTCATTTGGGAACCAATGAAGGAAGAC
CTAGTTTACCCGCTTGCTTCGTGCGGAACCCGTTCACTTAACCGAAAGCGCAAGCTTCT
CAGGTTTTGGAAGAGAGTGCCTGATAACACCTCTGAATTGAAAAACAAGACTCGGCTCC
TCAAAAGCAAT**TGTGGATTTCGACCTGCTAAAAGTCTCATCAGGGCAGCCAGCGACAGGCG**
CCTATCCACAACCACCTCGTTCTTCTTCAGAAGTCGCGGAGGTTCGGTTTGGGATCTAACCT
AGCGGTAAATCAAATTTTACTTTGTAAAACCTGCTTAGTTCAGACCTGACACCAGCCCC
Direct repeat { GTCGCTAATG**TTGAAGGAAGAAGTTGTGGGTGTAGTGCCCTGTCGCCGGGGCGCCTGATGA**
GACCAAGGCAGGTCGAAACACCTATTTGCTGGCGAGGTCCGGGAACCTTGT

Figure S2. Contig containing the sequences conserved in most natural hammerhead ribozymes in each polarity strand (red and light-blue colors) and a terminal direct repeat (yellow background).

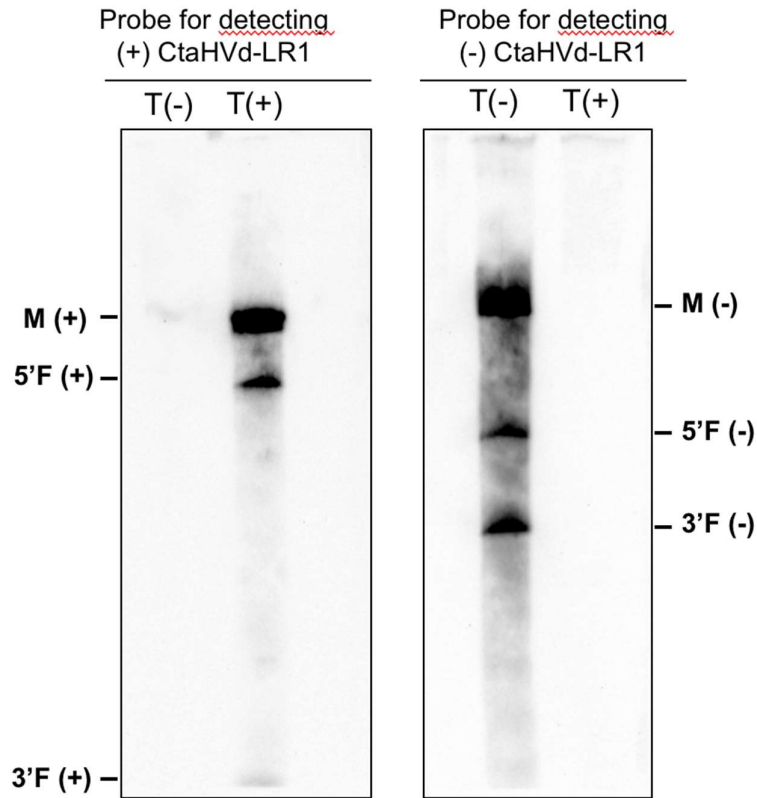


Figure S3. CtaHVd-LR1 (+) or (-) transcript RNAs detected by Northern-blot hybridization with equalized full-length digoxigenin-labeled riboprobes (left and right panel, respectively). Equal amounts of monomeric CtaHVd-LR1 (+) and (-) transcripts were loaded in lanes T(+) and T(-), respectively. The monomeric transcript (M) and the 5'F and 3'F fragments generated by the HHRzs are indicated.

	<div style="display: inline-block; width: 150px; border-bottom: 1px solid black; position: relative;"> ← Primer CH3 Rev Primer CH4 For → </div>	
CtaHVd-LR1_3	GTGCCCTGTCGCCGGGCGCCTGATCAGACCAAGGCAGGTCGAAACACCTATTGCTGGCG	60
CtaHVd-LR1_36C.....	60
CtaHVd-LR1_22A...	60
CtaHVd-LR1_17T.....	60
CtaHVd-LR1_23	60
CtaHVd-LR1_20A...	60
CtaHVd-LR1_24	60
CtaHVd-LR1_19	60
CtaHVd-LR1_18	60
CtaHVd-LR1_21	60
CtaHVd-LR1_11	60
CtaHVd-LR1_5	60
CtaHVd-LR1_10A...	60
CtaHVd-LR1_2A...	60
CtaHVd-LR1_33A.....	60
CtaHVd-LR1_32	60
CtaHVd-LR1_31	60
CtaHVd-LR1_14G.....	60
CtaHVd-LR1_9	60
CtaHVd-LR1_35	60
CtaHVd-LR1_12T.....	60
CtaHVd-LR1_6	60
CtaHVd-LR1_28	60
CtaHVd-LR1_8	60
CtaCtaHVd-LR1_34	60
CtaHVd-LR1_29A...	60
CtaHVd-LR1_27	60
CtaHVd-LR1_16	60
CtaHVd-LR1_30	60
CtaHVd-LR1_25	60
	***** * ***** **	

CtaHVd-LR1_3	AGGTCCGGGAACCTTTGTTATGTCAAAAGAGAGGGTAAATCGGGGAAACTCGCCTCCTAGA	120
CtaHVd-LR1_36C.....	120
CtaHVd-LR1_22C.....	120
CtaHVd-LR1_17A.....	120
CtaHVd-LR1_23C.....	120
CtaHVd-LR1_20C.....	120
CtaHVd-LR1_24C.....T.....	120
CtaHVd-LR1_19C.....	120
CtaHVd-LR1_18C.....	120
CtaHVd-LR1_21C.....	120
CtaHVd-LR1_11C.....A...	120
CtaHVd-LR1_5C.....	120
CtaHVd-LR1_10C.....	120
CtaHVd-LR1_2C.....G.....	120
CtaHVd-LR1_33A.....	120
CtaHVd-LR1_32C.....	120
CtaHVd-LR1_31C.....	120
CtaHVd-LR1_14C.....	120
CtaHVd-LR1_9C.....	120
CtaHVd-LR1_35C.....	120
CtaHVd-LR1_12C.....	120
CtaHVd-LR1_6C.....A.....	120
CtaHVd-LR1_28C.....	120
CtaHVd-LR1_8C.....T.....	120
CtaHVd-LR1_34C.....	120
CtaHVd-LR1_29	120
CtaHVd-LR1_27T.....	120
CtaHVd-LR1_16C.....	120
CtaHVd-LR1_30	120
CtaHVd-LR1_25C.....	120
	***** * ***** **	

	Primer CH1 Rev	Primer CH2 For	
CtaHVd-LR1_3	AATGAGGGCTCTGCTCTTACAGTATAATGGCGAGGTACCGTGCAGTTAGCAAGGGATTGC		180
CtaHVd-LR1_36T.....		180
CtaHVd-LR1_22A.....		180
CtaHVd-LR1_17A.....T.....		180
CtaHVd-LR1_23A.....		180
CtaHVd-LR1_20A.....		180
CtaHVd-LR1_24A.....A.....		180
CtaHVd-LR1_19A.....		180
CtaHVd-LR1_18A.....A.....		180
CtaHVd-LR1_21A.....		180
CtaHVd-LR1_11		180
CtaHVd-LR1_5		180
CtaHVd-LR1_10		180
CtaHVd-LR1_2		180
CtaHVd-LR1_33A.....		180
CtaHVd-LR1_32		180
CtaHVd-LR1_31A.....		180
CtaHVd-LR1_14		180
CtaHVd-LR1_9		180
CtaHVd-LR1_35		180
CtaHVd-LR1_12		180
CtaHVd-LR1_6		180
CtaHVd-LR1_28		180
CtaHVd-LR1_8		180
CtaHVd-LR1_34		180
CtaHVd-LR1_29C.....A.....		180
CtaHVd-LR1_27A.....		180
CtaHVd-LR1_16C.....		180
CtaHVd-LR1_30C.....A.....		180
CtaHVd-LR1_25C.....A.....		180
	***** * ***** ***** ** ***** *****		
CtaHVd-LR1_3	TAGCTTCTTTTCATTTGGGAAACCAATGAGAGAAGACCTAGTTTACCCGCTTGCTTCGTGC		240
CtaHVd-LR1_36C.....CCC.....CC..C..		240
CtaHVd-LR1_22AG.....		240
CtaHVd-LR1_17AG.....		240
CtaHVd-LR1_23AG.....		240
CtaHVd-LR1_20AG.....		240
CtaHVd-LR1_24		240
CtaHVd-LR1_19		240
CtaHVd-LR1_18		240
CtaHVd-LR1_21		240
CtaHVd-LR1_11AG.....		240
CtaHVd-LR1_5C.....AG.....		240
CtaHVd-LR1_10AG.....		240
CtaHVd-LR1_2AG.....		240
CtaHVd-LR1_33		240
CtaHVd-LR1_32AG.....		240
CtaHVd-LR1_31AG.....		240
CtaHVd-LR1_14AG.....		240
CtaHVd-LR1_9AG.....		240
CtaHVd-LR1_35AG.....T.....		240
CtaHVd-LR1_12AG.....T.....		240
CtaHVd-LR1_6AG.....T.....		240
CtaHVd-LR1_28AG.....		240
CtaHVd-LR1_8AG.....		240
CtaHVd-LR1_34AG.....		240
CtaHVd-LR1_29		240
CtaHVd-LR1_27		240
CtaHVd-LR1_16		240
CtaHVd-LR1_30T.....		240
CtaHVd-LR1_25		240
	***** ***** ** ***** *** ***** **		

CtaHVd-LR1_3	GGGAACCCGTTCACTTAACCGAAAGCGCAAGCTTCTCAGGTTTGGGAAGTGAGAGTCCTG	300
CtaHVd-LR1_36CC.....CC.....C.....A...T.....	300
CtaHVd-LR1_22A...T.....	300
CtaHVd-LR1_17	300
CtaHVd-LR1_23	300
CtaHVd-LR1_20A...T.....	300
CtaHVd-LR1_24	300
CtaHVd-LR1_19	300
CtaHVd-LR1_18	300
CtaHVd-LR1_21	300
CtaHVd-LR1_11G.....A...T.....	300
CtaHVd-LR1_5	300
CtaHVd-LR1_10A...T.....	300
CtaHVd-LR1_2	300
CtaHVd-LR1_33	300
CtaHVd-LR1_32A...T.....	300
CtaHVd-LR1_31	300
CtaHVd-LR1_14A.....	300
CtaHVd-LR1_9A...T.....	300
CtaHVd-LR1_35A...T.....	300
CtaHVd-LR1_12A...T.....	300
CtaHVd-LR1_6A...T.....	300
CtaHVd-LR1_28A...T.....	300
CtaHVd-LR1_8A...T.....	300
CtaHVd-LR1_34A...T.....	300
CtaHVd-LR1_29	300
CtaHVd-LR1_27	300
CtaHVd-LR1_16CC.....	300
CtaHVd-LR1_30	300
CtaHVd-LR1_25	300

CtaHVd-LR1_3	ATAATGCCTCTGAATTGAA-AAACAAAACCTCGGCTCCTCAAAAGCAATGTGGATTTCGAC	359
CtaHVd-LR1_36	...CC.....A.....G.....C.....	360
CtaHVd-LR1_22	...A.....G.....G.....	359
CtaHVd-LR1_17	...CA.....T.....	359
CtaHVd-LR1_23	...CA.....G.....	359
CtaHVd-LR1_20	...CA.....G.....G.....	359
CtaHVd-LR1_24	...C.....T.G.....	359
CtaHVd-LR1_19	...CA.....GT.....	359
CtaHVd-LR1_18	...CA.....G.....G.....	359
CtaHVd-LR1_21	...CA.....G.....A.....	359
CtaHVd-LR1_11	...CA.....T.TG.....G.....	360
CtaHVd-LR1_5	...CA.....G.....	359
CtaHVd-LR1_10	...CA.....G.....	359
CtaHVd-LR1_2	...CA.....T.....	359
CtaHVd-LR1_33	...CA.....G.....	359
CtaHVd-LR1_32	...CA.....G.....G.....	359
CtaHVd-LR1_31	...CA.....GT.....	359
CtaCtaHVd-LR1_14	...CA.....G.....	359
CtaHVd-LR1_9	...CA.....G.....	359
CtaHVd-LR1_35	...CA.....G.....A.....	359
CtaHVd-LR1_12	...CA.....G.....G.....	359
CtaHVd-LR1_6	...CA.....G.....	359
CtaHVd-LR1_28	...CA.....G.....	359
CtaHVd-LR1_8	...CA.....G.....	359
CtaCtaHVd-LR1_34	...CA.....G.....	359
CtaHVd-LR1_29	...CA.....T.....	359
CtaHVd-LR1_27	...CA.....G.....	359
CtaHVd-LR1_16	...CA.....G.....	359
CtaHVd-LR1_30	...CA.....G.....	359
CtaHVd-LR1_25	...CA.....G.....	359
	**** ***** * * * **** * ***** *****	

Accession	Sequence	Position
CtaHvd-LR1_3	CTGCTAAAAGTCTCATCAGGGCAGCCAGCGCAGGGCGCCTATCCACAACCACTCGTTCTT	419
CtaHvd-LR1_36	...C...	420
CtaHvd-LR1_22	...TTT...G...G...	419
CtaHvd-LR1_17	...A...	419
CtaHvd-LR1_23	...	419
CtaHvd-LR1_20	...	419
CtaHvd-LR1_24	...	419
CtaHvd-LR1_19	...C...	419
CtaHvd-LR1_18	...	419
CtaHvd-LR1_21	...	419
CtaHvd-LR1_11	...T...	420
CtaHvd-LR1_5	...	419
CtaHvd-LR1_10	...	419
CtaHvd-LR1_2	...	419
CtaHvd-LR1_33	...A...	419
CtaHvd-LR1_32	...G...	419
CtaHvd-LR1_31	...	419
CtaHvd-LR1_14	...	419
CtaHvd-LR1_9	...	419
CtaHvd-LR1_35	...A...	419
CtaHvd-LR1_12	...	419
CtaHvd-LR1_6	...	419
CtaHvd-LR1_28	...	419
CtaHvd-LR1_8	...	419
CtaHvd-LR1_34	...	419
CtaHvd-LR1_29	...A...	419
CtaHvd-LR1_27	...G...	419
CtaHvd-LR1_16	...C...	419
CtaHvd-LR1_30	...G...G...	419
CtaHvd-LR1_25	...G...	419

CtaHVd-LR1_3	CTTTGTAAACCTGCTTAGTTTCGGACCTGACACCAGCCCCGTCGCTAATGTTGAAGGAAG	536
CtaHVd-LR1_36CC.....A..CC..CC.A.....C.....	537
CtaHVd-LR1_22A.....A.....	537
CtaHVd-LR1_17	537
CtaHVd-LR1_23A.....A.....	537
CtaHVd-LR1_20A..G.....	537
CtaHVd-LR1_24A.....	537
CtaHVd-LR1_19	537
CtaHVd-LR1_18A.....A.....	537
CtaHVd-LR1_21A.....	537
CtaHVd-LR1_11A.....A.....	537
CtaHVd-LR1_5	T....A.....A.....	538
CtaHVd-LR1_10G...A...G...A.....G.....	536
CtaHVd-LR1_2A.....A.....	536
CtaHVd-LR1_33A.....A.....	536
CtaHVd-LR1_32G...A.....A.....	536
CtaHVd-LR1_31A.....A.....	536
CtaHVd-LR1_14A.....A.....	536
CtaHVd-LR1_9A....G.....A.....	536
CtaHVd-LR1_35A....G.....A.....	536
CtaHVd-LR1_12A....A.....A.....	536
CtaHVd-LR1_6A....G.....A.....	536
CtaHVd-LR1_28A.G.....A.....	536
CtaHVd-LR1_8A.....A.....	536
CtaHVd-LR1_34A.....A.....	536
CtaHVd-LR1_29G.....A.....	536
CtaHVd-LR1_27GG.....G...A.....	536
CtaHVd-LR1_16A.....C.....	536
CtaHVd-LR1_30G.....A.....	536
CtaHVd-LR1_25	536
	***** ** * * ***** ***** *****	
CtaHVd-LR1_3	AAGTTGTGGGTGTA	550
CtaHVd-LR1_36	551
CtaHVd-LR1_22	551
CtaHVd-LR1_17	551
CtaHVd-LR1_23	551
CtaHVd-LR1_20	551
CtaHVd-LR1_24	551
CtaHVd-LR1_19	551
CtaHVd-LR1_18	551
CtaHVd-LR1_21	551
CtaHVd-LR1_11	551
CtaHVd-LR1_5	552
CtaHVd-LR1_10	550
CtaHVd-LR1_2	550
CtaHVd-LR1_33	550
CtaHVd-LR1_32	550
CtaHVd-LR1_31	550
CtaHVd-LR1_14	550
CtaHVd-LR1_9	550
CtaHVd-LR1_35	550
CtaHVd-LR1_12	550
CtaHVd-LR1_6	550
CtaHVd-LR1_28	550
CtaHVd-LR1_8	550
CtaHVd-LR1_34	550
CtaHVd-LR1_29	550
CtaHVd-LR1_27	550
CtaHVd-LR1_16	550
CtaHVd-LR1_30	550
CtaHVd-LR1_25	550

Figure S4. Multiple-sequence alignment of full-length cDNAs of citrus transiently associated hammerhead viroid-like RNA 1 variants amplified with primer pairs CH1 Rev/CH2 For (from variant CtaHVd-LR1_2 to

CtaHVd-LR1_12), CH3 Rev/CH4 For (variants CtaHVd-LR1_14 to CtaHVd-LR1_24) and CH5 Rev/CH6 For (variants CtaHVd-LR1_25 to CtaHVd-LR1_36). Names of the sequence variants and nucleotide positions in the alignment are reported on the left and the right, respectively. Regions covered by reverse (Rev) and forward (For) primers are marked by arrows (blue and green, respectively). Nucleotide identity and gaps with respect to the reference variant are indicated by dots and dashes, respectively. Regions involved in the formation of (+) and (-) hammerhead structures are in red and blue, respectively, with the nucleotides conserved in most natural hammerhead structures on a grey background. The reference variant (CtaHVd-LR_3) reported at the top shares the highest identity with the consensus sequence of the alignment, from which it differs in six nucleotides not located in the conserved HHRzs motifs.

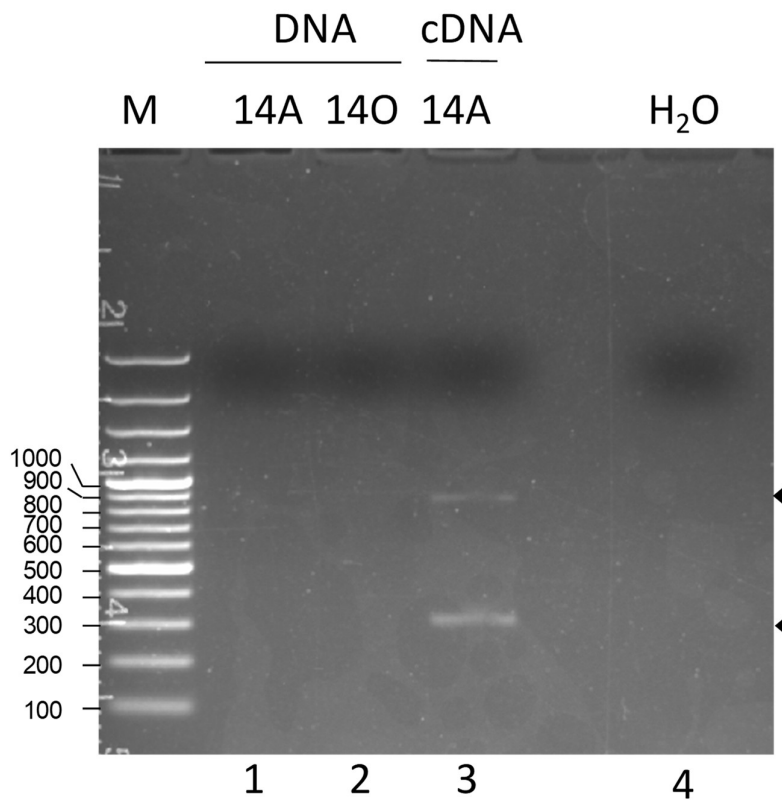


Figure S5. PCR and RT-PCR assays using DNA (lane 1) and RNA (lane 3) preparations from isolate 14A, respectively. A DNA preparation from the citrus isolate 14O (lane 2) and a mix in which water replaced DNA (lane 4) were used as negative controls for the PCR assay. Molecular Marker (M) is 100 bp ladder (New England Biolabs). Arrowhead on the left indicate the HVd-LR1 amplicon of 304 nt expected to be amplified by RT-PCR from isolate 14A and a larger amplicon deriving from the amplification of a larger than unit CtaHVd-LR1 cDNA.