

Article

Optimization of a Protocol for Launching Grapevine Infection with the Biologically Active cDNA Clones of a Virus

Mehdi Shabanian^{1,*}, Caihong Li ¹, Ali Ebadi ², Valerian Dolja³ and Baozhong Meng ¹

¹ Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1, Canada; caihong@uoguelph.ca (C.L.); bmeng@uoguelph.ca (B.M.)

² Department of Horticulture, College of Agriculture and Natural Resources, University of Tehran, Karaj 31587–11167, Iran; aebadi@ut.ac.ir

³ Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA; doljav@oregonstate.edu

* Correspondence: shabania@uoguelph.ca

Supplemental Tables:

Citation:Shabanian, M.; Li, C.; Ebadi, A.; Dolja, V.; Meng, B. Optimization of a Protocol for Launching Grapevine Infection with the Biologically Active cDNA Clones of a Virus. *Pathogens* **2023**, *12*, 1314. <https://doi.org/10.3390/pathogens1211314>

Academic Editors: Kathleen Heferon and Srividhya Venkataraman

Received: 6 October 2023

Revised: 25 October 2023

Accepted: 30 October 2023

Published: 3 November 2023



Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Table S1. Agar media for all grape stages OH, GS1, GS2 and GS3.

Media Stage	OH medium.	GS1	GS2	GS3
Ingredients/1 L	pH: 5.7	pH: 5.3	pH: 5.3	pH: 5.3
M & S salts	3.22 g	3.22 g	3.22 g	3.22 g
Thiamine	0.8 mL (0.5 mg/mL)			
Inositol	0.100 g	0.1 g	0.025 g	0.025 g
Sucrose	7 g	20 g	15 g	12.5 g
BAP	–	2 mL (0.5 mg/mL)	4 mL (0.5 mg/mL)	–
NaH ₂ PO ₄	0.170 g	–	0.05 g	–
Adenine sulfate	0.08 g	–	–	–
IAA	–	–	0.5 mL (1mg/mL)	1 mL (1mg/mL)
Agar	3 g	2 g	2 g	1 g
Gelrite	–	1.13 g	1.2 g	1 g
Cefotaxime	0.2 g	0.2 g	0.2 g	0.2 g

Table S2. Primers were used in this study.

Primers	Sequences	Amplicon (bp)	Target Gene
LR3CP107F	5'-TCTTAAARTAYGTTAAGGACGG-3'	836	CP of GLRaV-3
LR3CP942R	5'-CTAGTTCITTTTGCAATAGTTG-3'		
LR3_14117F	5'-GTGCCGGAATCTGAGACTCT-3'	211	CP of GLRaV-3
LR3_14327R	5'-GCCCATAACCTTCTTACACAGC-3'		
LR3_16289F	5'-GTTTCGGAGACGTATAACATC-3'	951	ORF8 &9 of GLRaV-3
LR3_17240R	5'-CTAATCCTCGATTCTCCCCTGAC-3'		
LR3_16896F	5'-CGCTTAAAACGAACGATCAC-3'	344	ORF9 of GLRaV-3
LR3_17240R	5'-CTAATCCTCGATTCTCCCCTGAC-3'		
LR2-C-F	5'-AAGGAATACTTAGGCGCCGAC-3'	1458	3' end region including GFP of GFP-tagged GLRaV-2
LR2-C-R	5'-AGGAGCCCCTTTCTGCACCAA-3'		
eGFP-F	5'-AAAGGTACCGTGAGCAAGGGCGAGGA-3'	720	eGFP
eGFP-R	5'-AAAGGATCCTTACTTGTGACGCTCGTCCATGC-3'		
ACT-1021F	5'-ATCAGGAAGGACCTCTATGG-3'	206	Actin 1 of grapevine
ACT-1226R	5'-ATCCACATCTGCTGGAAGG-3'		