PHYTOREOVIRUS-LIKE SEQUENCES ISOLATED FROM SALIVARY GLANDS OF THE GLASSY-WINGED SHARPSHOOTER *HOMALODISCA VITRIPENNIS* (HEMIPTERA: CICADELLIDAE)

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ABSTRACT

The salivary glands of the Glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* Germar 1821, (syn. *H. coagulata*, Hemiptera: Cicadellidae) were collected and used to produce a cDNA library. Examination by BLASTX analyses identified 2 viral sequences, one a 610-base pair fragment and a second 839-base pair fragment, both of which had significant homology to viruses within the genus *Phytoreovirus*. Resequencing of the fragments confirmed sequence validities. These sequences were used for *in silico* protein translation and BLASTP analysis confirming the established homology. While the GWSS is the primary vector of Pierce's disease of grapes, this is the first report that GWSS may be a vector of a phytoreoviruses. Phylogenetic and homology comparisons with BLASTX, BLASTP, and PAUP analyses indicated that the viral sequences isolated from GWSS were closely related to the viruses in the Family *Reoviridae*, Genus *Phytoreovirus*, specifically Rice Dwarf *Phytoreovirus* (RDV). RDV is the only plant reovirus that is not limited to the phloem. Phytoreoviruses are transmitted in a propagative manner by cicadellid leafhoppers (Hemiptera: Cicadellidae), which acquire and transmit them during feeding. Phytoreoviruses have been reported from *Agallian*, *Agalliopsis*, *Nephotettix*, and *Recilia*, genera of leafhoppers, with evidence for transovarial transmission. The GWSS, although considered to feed primarily from the xylem, ingests from other plant tissues, such as the phloem and mesophyll during probing similar to other leafhoppers. The feeding behavior and wide host range of the GWSS provides an overlapping condition for these two organisms, leafhopper and virus. GWSS will feed from grasses as a transitory host, and on herbaceous and woody plants as primary hosts, which may favor the acquisition and transmission of *Phytoreovirus* by this leafhopper. Monitoring for an increase of *Phytoreovirus* spread in graminaceous crops that are in proximity to vineyards or tree crop orchards, where GWSS occurs, such as in southern California, will provide a better understanding of the potential role of the GWSS as a disease vector in the spread of phytoreoviruses and other plant pathogens. The sequences have been deposited in NCBI database under the accession numbers (EF058280) for GWSS-V1, WHSg013C11 and (EF058281) for GWSS-V2, WHSg024H02.

Key Words: *Homalodisca coagulata*, *H. vitripennis*, leafhopper, *Phytoreovirus*, Reoviridae, Rice Dwarf Virus (RDV), Wounded Tumor Virus (WTV)

RESUMEN

Dos fragmentos de 610 y 839 pares de bases fueron aislados apartir de una genoteca de expresión derivada de las glandulas salivales del cucarrón de las alas cristalinas (GWSS), *Homalodisca vitripennis*, Germar 1821 (syn. *H. coagulata*) el cual es vector de la enfermedad de Pierce de las uvas. Los resultados de alineamiento utilizando BLASTX, BLASTP y el analisis filogenético utilizando PAUP indicaron que los fragmentos de DNA estaban relacionado de manera mas cercana a viruses en la familia *Reoviridae*, género *Phytoreovirus*, y específicamente a los virus del enanismo del arroz (RDV) y al virus del tumor de las grietas (WTV). El cucarrón de las alas cristalinas es un saltahoja que se alimenta no solo del xilema sino tambien del floema y del mesófilo. Saltahojas del género *Agallian*, son los principales vectores de WTV, el cual infecta el floema de plantas dicotiledoneas tumoraciones en las hojas y en las raices. WTV es transmitido por saltahojas y es el único reovirus que es capaz de infectar tanto tejidos del xilema como del floema. El comportamiento alimentario del GWSS y su amplio rango de hospederos que incluye pastos y plantas herbaceas y leñosas podria proveer la interacción entre estos dos organismos facilitando la adquisición y tranmisión de fitoreovirus por el GWSS. Un adecuado monitoreo de el incremento en la expansión de reoviruses en cultivos de gramineas asociados al los viñedos en donde GWSS ocurre en regiones tales como el sur de California, y en general el sur de los Estados Unidos, podría proveer un mejor entendimiento

del papel del GWSS como vector de fitoreovirus y otros patógenos de plantas. Las sequencias se depositaron en la base de datos NCBI con los siguientes números de identificación: (EF058280) para GWSS-V1, WHSg013C11 y (EF058281) para GWSS-V2, WHSg024H02.

Translation provided by the authors.

Annotation of a cDNA salivary gland library of the Glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* Germar 1821 (Hemiptera: Cicadellidae) (Takiya et al. 2006, syn. *H. coagulata*), resulted in the discovery and validation of 2 viral sequences, one a 610-base pair fragment and a second 839-base pair fragment, both of which had significant homology to viruses within the genus, *Phytoreovirus*. Leafhoppers are the second most serious pests in agriculture and are the only known vectors of *Phytoreovirus*, such as wound tumor virus, WTV, which occurs in North America and infects the phloem of dicotyledonous plants (Black 1945). The GWSS is an important agricultural pests of grapes and fruit crops as the primary vector of Pierce's disease, caused by a bacterial pathogen of plants that occurs across the southern United States. Phylogenetic and homology comparisons with BLASTX, BLASTP, and PAUP (Swofford 2003) analyses indicated the viral sequences were closely related to the family, *Reoviridae*, genus *Phytoreovirus*, specifically Rice Dwarf Virus (RDV) (Hillman et al. 1991; Suzuki et al. 1999). RDV is the only plant reovirus that is not limited to the phloem (Omura & Mertens 2005) and is the only virus member that must pass through an insect vector prior to becoming infectious (Johnson 2003). *Phytoreovirus* are large, complex viruses containing segmented double-stranded RNA genomes that infect plant hosts through their transmission by leafhoppers (Van Regenmortel et al. 2000). Phytoreoviruses are not transmitted by mechanical inoculation, contact between plants, nor through seed or pollen, but are acquired and transmitted in a propagative manner by cicadellid leafhoppers (Hemiptera: Cicadellidae) during feeding. Phytoreoviruses have been reported from *Agallian*, *Agalliopsis*, *Nephotettix*, and *Recilia*, genera of leafhoppers, with evidence for transovarial transmission (Omura and Mertens 2005). The transmission of phytoreovirus is normally by phloem-feeding leafhoppers. While the GWSS is considered to feed primarily from the xylem, during probing leafhoppers also sample from other plant tissues such as the phloem and mesophyll (Backus & Hunter 1989; Hunter & Backus 1989).

The *Phytoreovirus*, WTV, has been shown to infect more than 20 different families of dicotyledonous plants like crimson clover (*Trifolium incarnatum*), sweet clover (*Melilotus officalis*), and cultivated sorrel (*Rumex acetosa* (Black 1945). While WTV has been reported to occur in North America (Black 1972) and WTV-susceptible crops, such as clovers, are recommended as cover crops to reduce soil erosion in grape vineyards (Stimson & O'Conner 2005), RDV has yet to be found in South or Central America (Lee et al. 1997). The combination of leafhopper feeding behaviors (feeding on grasses as transitory hosts, and on herbaceous and woody plants as primary hosts), with the wide host-plant range of the GWSS (Hoddle et al. 2003; Hoddle 2004), provides overlapping conditions that may favor the acquisition and transmission of *Phytoreovirus* by the GWSS.

Materials and Methods

Library Construction

Insects for library construction were collected off several citrus trees in Kern County, California. Dissected salivary glands from 50 adult GWSS were used in the construction of an expression library. Tissues were ground in liquid nitrogen and total RNA was extracted using guanidinium salt-phenol-chloroform procedure as previously described by Strommer et al. (1993). Poly (A) +RNA was purified using Micromole (A) Pure™ according to the manufacturer's instructions (Ambion, Austin, TX, USA). A directional cDNA library was constructed in Lambda Uni-ZAP® XR vector using Stratagene's ZAP-cDNA Synthesis Kit (Stratagene, CA, USA). The resulting DNA was packaged into Lambda particles using Gigapack® III Gold Packaging Extract (Stratagene, CA, USA). An amplified library was generated with a titer of 1.0×10^9 plaque-forming units per mL. Mass excision of the amplified library was carried out using Ex-Assist® helper phage (Stratagene, CA, USA). An aliquot of the excised, amplified library was used for infecting XL1-Blue MRF' cells and subsequently plated on LB agar containing 100 µg/mL ampicillin. Bacterial clones containing excised pBluescript SK(+) phagemids were recovered by random colony selection.

Sequencing of Clones

pBluescript SK (+) phagemids were grown overnight at 37°C and 240 rpm in 96-well culture plates containing 1.7 mL of LB broth, supplemented with 100 µg/mL ampicillin. Archived stocks were prepared from the cell cultures using 75 µL of a LB-amp-glycerol mixture and 75 µL of cells. These archived stocks are held at the United States Horticultural Research Lab (USHRL) in an ultra low temperature freezer set at -80°C. Plasmid DNA was extracted with the Qiagen

9600 liquid handling robot and the QIAprep 96 Turbo miniprep kit according to the recommended protocol (QIAGEN Inc., CA, USA). Sequencing reactions were performed with the ABI PRISM® BigDye™ Primer Cycle Sequencing Kit (Applied Biosystems, CA, USA) along with a universal T3 primer. Reactions were prepared in 96-well format using the Biomek2000™ liquid handling robot (Beckman Coulter, Inc., CA, USA). Sequencing reaction products were precipitated with 70% isopropanol, resuspended in 15 µL sterile water and loaded onto an ABI 3700 DNA Analyzer (Applied Biosystems, CA, USA).

Sequence Verification and Cloning

Base confidence scores were designated using TraceTuner® (Paracel, Pasadena, CA, USA). Low-quality bases (confidence score <20) were trimmed from both ends of sequences. Quality trimming, vector trimming and sequence fragment alignments were executed using Sequencher® software (Gene Codes, MI, USA). Sequences less than 100 nucleotides in length after both vector and quality trimming was completed were excluded from the analysis. Additional ESTs that corresponded to vector sequences were removed from the dataset. The potential status of the gene was determined based on BLAST homology searches using the National Center for Biotechnology Information BLAST server (http://www.ncbi.nlm.nih.gov) with the sequence comparisons made to protein databases (BLASTX, BLASTP). To estimate the number of genes represented in the library and the redundancy of specific genes, ESTs were assembled into "contigs" using Sequencher®. Contig assembly parameters were set using a minimum overlap of 50 bases and 95% identity match. Vector and low-quality sequence were trimmed and the sequences filtered for a minimum length (200 bp), producing 8884 high-quality ESTs. These ESTs were analyzed with the Sequencher® assembly program to identify those representing redundant transcripts. Two clones were identified for further analysis. To ensure sequence accuracy clones were bidirectionally sequenced 3 times. The resulting sequences were assembled into a contig using Sequencher® with the same parameters used above. The resulting contigs were run through the National Center for Biotechnology Information BLAST server (http://www.ncbi.nlm.nih.gov) using BLASTX, TBLASTX, TBLASTN.

Computer Analysis

The amino acid sequence of the clones were calculated with the 'Translate' program on the ExPASy server (http://au.expasy.org). The resulting sequences were then analyzed by the National Center for Biotechnology Information BLAST server (http://www.ncbi.nlm.nih.gov) and BLASTP (Schäffer et al. 2001). Multiple sequence alignments of predicted *Phytoreovirus* amino acid sequences were performed with T-Coffee (Notredame et al. 2000) and ClustalX version 1.81 algorithm (Thompson et al. 1997). Guide trees were generated using neighbor-joining (NJ) methodology. NJ analyses were performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.010) (Swofford 2003). NJ estimates of the Phytoreovirus sequences were obtained by estimating distance parameters in PAUP* (Swofford 2003). NJ bootstrap analyses of 1000 replicates were performed on each data set using a heuristic search to identify the most optimal tree. Analyses were unrooted. Tree branch probabilities added by a Bayesian approach to phylogenetic reconstruction implemented using MRBAYES v3.1.1 (Huelsenbeck & Ronquist 2001). Sequences used in the phylogenetic analysis encompassed Core protein P3 from RDV (accession Q98630), Core capsid protein Rice gall dwarf virus, RGDV (accession BAA02917), RDV RNA-dependent RNA polymerase (accession BAA14222), RDV RNA-directed RNA polymerase (accession Q02119), Nonstructural WTV P7 (accession P13092), Structural WTV P9 (accession P12326), RGDV Nonstructural VP10 (accession P29078), RDVA minor core P5 (accession P14583), RDV minor core P5 (accession Q85437), WTV polypeptide P5 (accession AAA48499), WTV outer coat P5 (accession P12366), WTV structural P6 (accession P12325), WTV P6 (accession CAA32438), RDV structural P7 (accession Q85448), RGDV outer capsid P8 (accession P29077), WTV outer capsid P8 (accession P17380), RDV outer capsid P8 (accession Q85451), RDV nonstructural P4 (accession Q85436), RDV outer capsid P2 (accession O55519), WTV nonstructural P11 (accession P13094), WTV nonstructural P12 (accession P13278), RDV nonstructural P11 (accession Q85442), RGDV nonstructural VP9 (accession P23628), RDV nonstructural P6 (accession P29249) (Fig. 1).

Results

Phylogenetic and homology comparisons with BLASTX, BLASTP and PAUP (Altschul et al. 1997; Schäffer et al. 2001; Swofford 2003) analyses indicated the 2 sequences isolated from GWSS salivary glands were closely related to the family *Reoviridae*, genus *Phytoreovirus*, specifically Rice Dwarf Virus (RDV). The GWSS-V1 sequence shared a 47% amino acid identity to the Minor core P5 protein of the Rice dwarf *Phytoreovirus*, with an E-value of 9e-20 (Table 1). GWSS-V2 sequence shared 40% amino acid identity to the non-structural S9 Rice dwarf virus, with an E-value of 3e-49 (Table 2). Sequences with significant amino acid identity, along with other members of the Reoviridae, were downloaded for phy-

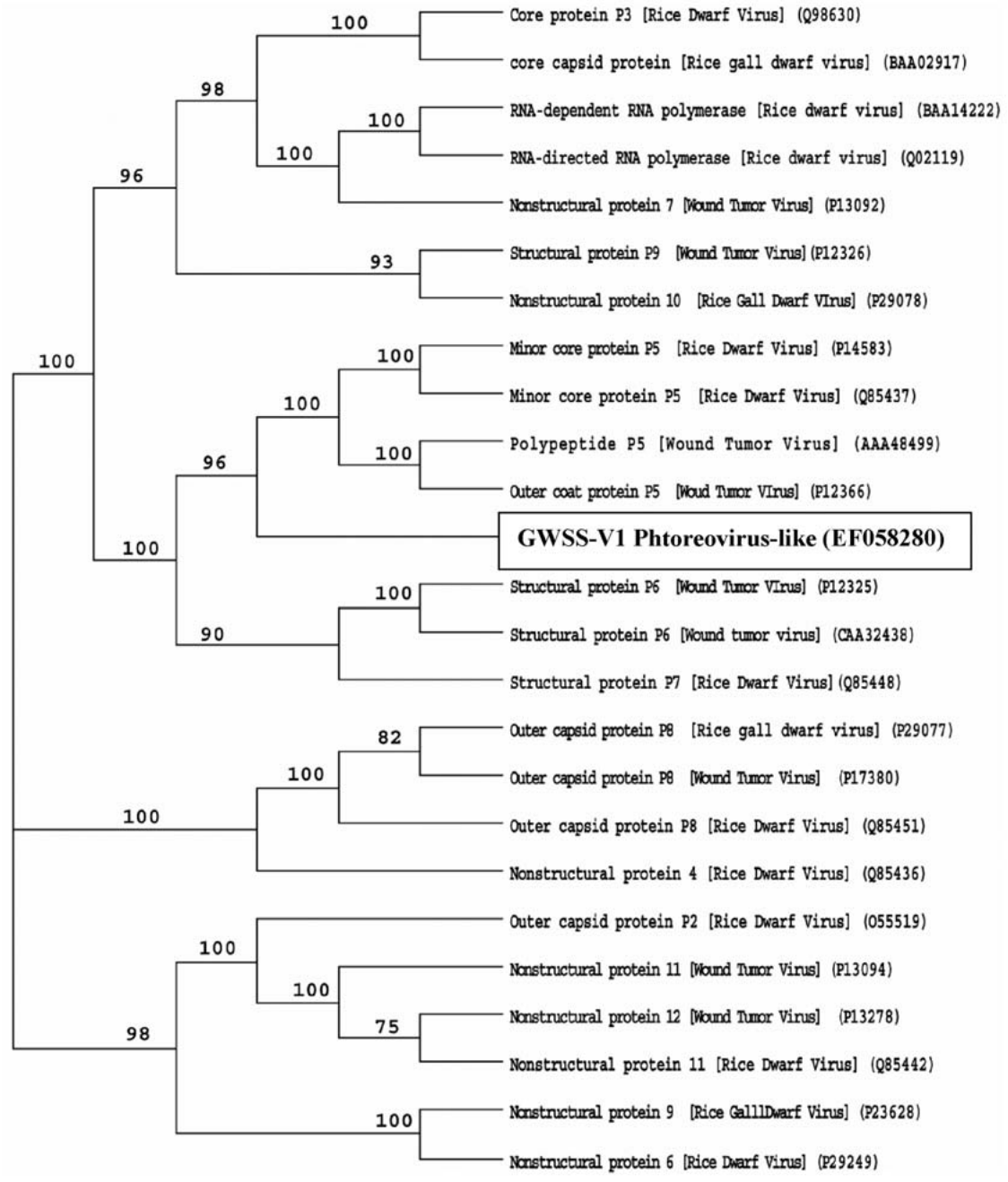

Fig. 1. Phylogenetic analysis of *Phytoreovirus* amino acid sequences. GWSS-V1 phytoreovirus-like sequence forms a clade with the P5 proteins with significant identity to WTV and RDV. Multiple sequence alignments of predicted phytoreovirus amino acid sequences were performed with ClustalX version 1.81 algorithm (Thompson et al. 1997). Guide trees were generated with neighbor-joining (NJ) and Bayesian methods. NJ analyses were performed with PAUP* (Phylogenetic Analysis Using Parsimony) version 4.010) (Swofford 2003). NJ estimates of the Phytoreoviruses were obtained by estimating distance parameters in PAUP* (Swofford 2003). NJ bootstrap analyses of 1000 replicates were performed on each data set by a heuristic search to identify the most optimal tree. Analyses were unrooted.

TABLE 1. TRANSLATED AMINO ACID IDENTITY SCORES FOR HOMOLOGY SEARCH TO GWSS-*PHYTOREOVIRUS*-LIKE SEQUENCE, TOP 4 RETURNED MATCHES. BLASTX ANALYSES FROM NCBI WEBSITE (HTTP://WWW.NCBI.NLM.NIH.GOV/BLAST/) (GWSS-V1 ACCESSION NO. EF058280).

Virus accession	% Amino acid identity	BLASTP[1]
Rice dwarf virus (Q85437) Minor core P5	47	$9e^{-20}$
Rice dwarf virus (P14583) Minor core (VP5_RDV)	47	$1e^{-19}$
Rice Gall Dwarf Virus (Q12NZ7) RDGV	40	$3e^{-19}$
P5Wound tumor virus (P12366) Outer coat VP5	44	$3e^{-18}$

logenetic comparison to the GWSS phytoreovirus-like sequences. In both analyses the highest degree of identity to the GWSS-derived sequences was to RDV (Tables 1 and 2, Fig. 1). Using maximum parsimony (branch-and-bound search method), the phylogenetic tree of the translated amino acid sequence for GWSS-V1 was equally related to either RDV or WTV (Fig. 1). Amino acid alignment of GWSS-V2 resulted in a significantly greater identity to RDV proteins (Fig. 2, Table 2).

DISCUSSION

Results presented provide evidence for the presence of a *Phytoreovirus* in the tissues of the salivary glands of the GWSS, strongly suggesting that the GWSS is feeding on plants containing *Phytoreovirus*, and that the virus is able to move through the tissues of the GWSS to enter the salivary glands as in the manner of a virus-vector interaction. While there is no direct evidence of disease transmission, leafhoppers are the normal vectors of *Phytoreovirus*. These results also suggest that the GWSS may be causing more economic damage than previously thought by also being a vector of these plant pathogens. While the GWSS *Phytoreovirus*-like sequences (GWSS-V1 and V2) are most closely related to RDV, they might very well be part of the genome of a new member within the phytoreoviruses, or an undescribed variant of WTV, taking into consideration that RDV has not yet been reported to occur within the USA. This is the first report that the GWSS may be a vector of *Phytoreovirus*, and warrants closer examination. Virus acquisition and transmission may be occurring due to a combination of the GWSS feeding behavior (feeding on grasses as transitory hosts, and on herbaceous and woody plants as primary hosts) and wide host range (Hoddle et al. 2003, Hoddle 2004) which overlaps with *Phytoreovirus* host plants (Figs. 3 and 4). The insects from which the GWSS salivary gland library was produced were collected in Kern County, CA, in a region where grapes are grown. WTV-susceptible crops, such as clovers, are sometimes recommended as cover crops to reduce soil erosion in grape vineyards by the State Water Resources Control Board of California (Stimson and O'Conner 2005) creating overlapping ranges for the GWSS and phytoreoviruses. Three common WTV host plants, Madagascar periwinkle (*Catharanthus roseus*), New Zealand Spinach (*Tetragonia tretragoniodes*), and Cardinal flower (*Lobelia cardinalis*), are found throughout California and Florida within the normal GWSS range (Figs. 2 and 3) (USDA, NRCS 2005, http://plants.usda.gov/checklist.html). The presence of both *Phytoreovirus* and the GWSS in these regions may indicate that *Phytoreovirus* are being transmitted by the GWSS to other susceptible crops through nonspecific feeding such as during the production of sample probes during host plant selection (Backus & Hunter 1989; Hunter & Backus 1989). A real concern to growers would be if WTV, or another related virus, can be acquired by the GWSS which in turn would then transmit it to a wider range of host plants, ultimately resulting in increased economic losses, similar to the events leading to the extensive spread of Pierce's disease in grapes. Monitoring for increased incidence of WTV, RDV and/or other leafhopper transmitted viruses within graminaceous

TABLE 2. TRANSLATED AMINO ACID IDENTITY SCORES FOR HOMOLOGY SEARCH TO GWSS-V2 *PHYTOREOVIRUS* SEQUENCE, TOP 4 MATCHES. BLASTX ANALYSES FROM NCBI WEBSITE (HTTP://WWW.NCBI.NLM.NIH.GOV/BLAST/) (GWSS-V2 ACCESSION NO. EF058281).

Virus accession	% Amino acid identity	BLASTP[1]
Non-structural protein S9 Rice dwarf virus (Q85450)	40	$3e^{-49}$
Non-structural protein Pns9, Rice dwarf virus (P17381)	40	$4e^{-49}$
Non-structural protein S9 Rice dwarf virus (AAP92807.1)	40	$6e^{-49}$
Non-structural protein S9 Rice dwarf virus (NP_620535)	40	$1e^{-48}$

GWSS-V2 (accession no EF058281)	Len: 249 aa
RDV Ns S9 Rice dwarf virus, AAP92807.1	Len: 351 aa
RDVA Ns Pns9, VP9, Q85450	Len: 351 aa
RDV VP9 Ns Pns9, P17381	Len: 351 aa
RDV_Ns Rice dwarf virus, NP_620535.1	Len: 351 aa
RDVO VP90 Ns Pns9 Q85446	Len: 351 aa
WTVNJ Structural prot.P9 Capsomere, P31611	Len: 313 aa
WTV_Structural_protein_P9_Capsomere, P12326	Len: 313 aa
RGDV_Ns Pns10 Rice gall dwarf virus, ABC75538.1	Len: 320 aa
RGDV_Rice gall dwarf virus, AAS66755.1	Len: 320 aa
VP10_RGDV Ns Pns10, P29078	Len: 320 aa

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GWSS-V2                                 --QKLQDGTAIKRINDAIQIFQKYQTGEVSKSSSNHLNQLRNIRMSVGLAWPVILKYCFL
RDV_Ns_S9_Rice_dwarf_virus              -MGKLQDGIAIKRINDAITTFKNYKLGELEQGGSMAINTLSNVRAHVGLAWPAILRNCLI
RDVA_Ns_Pns9_VP9                        -MGKLQDGIAIKRINDAITTFKNYKLGELEQGGSMAINTLSNVRAHVGLAWPAILRNCLI
RDV_VP9_Ns_Pns9                         -MGKLQDGIAIKRINDAITTFKNYKLGELEQGGSMAINTLSNVRAHVGLAWPAILRNCLI
RDV_Ns_Rice dwarf_virus                 -MGKLQDGIAIKRINDAITTFKNYKLGELKQGGSMAINTLSNVRAHVGLAWPAILRNCLI
RDVO_VP90_Ns_Pns9                       -MGKLQDGIAIKRINDAITTFKNYKLDELEQGGSMAINTLSNVRAHVGLAWPAILRNCLI
WTVNJ_Structural_protein_P9             MSGKIQDGVAIRRMSDAILFFTNYTSRNLIDQRDITLSTLHTIRRNLGTCWSIALLNCWN
WTV_Structural_protein_P9               MSGKIQDGVAIRRMSDAILFFTNYTSRNLIDQRDITLSTLHTIRRNLGTCWSIALLNCWN
RGDV_Ns_Pns10_Rice gall dwarf virus     MAGKLQDGVAIAKIKETINLFCEYSFGDLVNNRREIVGRVHDARKNAALAWPDLIMNCFL
RGDV_Rice gall dwarf virus              MAGKLQDGVAIAKIKETINLFCEYSFGDLVNNRREIVGRVHDARKNAALAWPDLIMNCFL
VP10_RGDV_Ns_Pns10                      MAGKLQDGVAIAKIKETINLFCEYSFGDLVNNRREIVGRVHDARKNAALAWPDLIMNCFL
                                           *:*** ** ::.::*  * :*   :: .     :. :    *   . .*.  :  *

GWSS-V2                                 HVSSHCGVVRFLTDLACTVKVGVFPIISSIGDFDPFDDVGLIFSKSCIALKISDSSFLTP
RDV_Ns_S9_Rice_dwarf_virus              HTSSHLGFMKFMIDIATTWKVGAFTLLGSVGDEDPFTDVDLIYTKTCLHLGLKDNDFLQF
RDVA_Ns_Pns9_VP9                        HTSSHLGFMKFMIDIATTWKVGAFTLLGSVGDEDPFTDVDLIYTKTCLHLGLKDNDFLQF
RDV_VP9_Ns_Pns9                         HTSSHLGFMKFMIDIATTWKVGAFTLLGSVGDEDPFTDVDLIYTKTCLHLGLKDNDFLQF
RDV_Ns_Rice dwarf_virus                 HTSSHLGFMKFMIDIATTWKVGAFTLLGSVGDEDPFTDVDLIYTKTCLHLGLKDNDFLQF
RDVO_VP90_Ns_Pns9                       HTSSHLGFMKFMIDIATTWKVGAFTLLGSVGDEDPFTDVDLIYTKTCLHLGLKDNDFLQF
WTVNJ_Structural_protein_P9             ETSSHAGVMRFILDIAFSLRFGDFTMLGACGNVDPFDDAGQIFLKSCKATGRNDSCFLTP
WTV_Structural_protein_P9               ETSSHAGVMRFILDIAFSLRFGDFTMLGACGNVDPFDDAGQIFLKSCKATGRNDSCFLTP
RGDV_Ns_Pns10_Rice gall dwarf virus     HSASHYGVVKFLLDIALSTRFGDFTLLGVSSQNYPFYDLHVVMTKAFCNLDFAKDEYLMI
RGDV_Rice gall dwarf virus              HSASHYGVVKFLLDIALSTRFGDFTLLGVSSQNYPFYDLHVVMTKAFCNLDFAKDEYLMI
VP10_RGDV_Ns_Pns10                      HSASHYGVVKFLLDIALSTRFGDFTLLGVSSQNYPFYDLHVVMTKAFCNLDFAKDEYLMI
                                        .  :** *.::*: *:* : :.* *.::.  .:   ** *    :   *:       .. :*

GWSS-V2                                 PDSFASKLTLFFSAMSVNARVET-DMLHEIEDRYTYRTGVIGELLSVYMNAT-LNL---D
RDV_Ns_S9_Rice_dwarf_virus              PEEFAYEANSFLEAQSMNARVDLLTGVHNIEDKYVFRMQSISKFLKAYYTAS-EDVAYLT
RDVA_Ns_Pns9_VP9                        PEEFAYEANSFLEAQSMNARVDMLTGVHNIEDKYVFRMQSISKFLKAYYTAS-EDVAYLT
RDV_VP9_Ns_Pns9                         PEEFAYEANSFLEAQSMNARVDMLTGVHNIEDKYVFRIESISKFLKAYYTAS-EDVAYLT
RDV_Ns_Rice dwarf_virus                 PEEFAYEANSFLEAQSMNAKVDMLTGVHNIEDKYVFRMQSISKFLKAYYTAS-EDVAYLT
RDVO_VP90_Ns_Pns9                       PEEFAYEANSFLEAQSMNARVDMLTGVHNIEDKYVFRMQSISKFLKAYYTAS-EDVAYLT
WTVNJ_Structural_protein_P9             SDNFGYYLVSFLNKEQLKCVVDMNVGIHNIEDIYVTRMESIMEFIYYYYTESGRDV---V
WTV_Structural_protein_P9               SDNFGYYLVSFLNKEQLKCVVDMNVGIHNIEDIYVTRMESIMEFIYYYYTESGRDV---V
RGDV_Ns_Pns10_Rice gall dwarf virus     NDSFSSMMSAFLDEEGVHSAMSMELGIHDIEDRFVLRTKRLFYIIHEYHMSL-DEIEPWL
RGDV_Rice gall dwarf virus              NDSFSSMMSAFLDEEGVHSAMSMELGIHDIEDRFVLRTKRLFYIIHEYHMSL-DEIEPWL
VP10_RGDV_Ns_Pns10                      NDSFSSMMSAFLDEEGVHSAMSMELGIHDIEDRFVLRTKRLFYIIHEYHMSL-DEIEPWL
                                         :.*.      *:.   ::. :.     :*:*** :. *    :  ::  *      ::

GWSS-V2                                 DWVVNLD-----ELSQPT-RAQMLTLLAQNRNMISNEIRNYVNKNLDHPSEHLAAVAKKY
RDV_Ns_S9_Rice_dwarf_virus              GFIKPDDSRDSILNAELL-KAQVTSEVLRVRNLITTKIQKYINLYEDSQLPHFRQAALSY
RDVA_Ns_Pns9_VP9                        GFIKPDDSKDSILSAELL-KAQVTSEVLRVRNLITTKIQKYINLYEDSQLPHFRQAALSY
RDV_VP9_Ns_Pns9                         GFIKPDGSKESILSAELL-KAQVTSEVLRVRNLITTKIQQYINLYEDSQLPHFRRAALSY
RDV_Ns_Rice dwarf_virus                 GFIKPDDSKDSILNAELL-EAQVTSEVLRVRNLITTKIQKYINLYEDSQLPHFRQAALSY
RDVO_VP90_Ns_Pns9                       GFIKPDDSKDSILSAELL-KAQVTSEVLRVRNLITTKIQKYINLYEDSQLPHFRQAALSY
WTVNJ_Structural_protein_P9             NWLEKLESADAGLAAHAKSKRLMRAEIDLIRREILERTRLFINSNRNSFHDHHRELVRRY
WTV_Structural_protein_P9               NWLEKLESADAGLAAHAKSKRLMRAEIDLIRREILERTRLFINNNRNSFHDHHRELVRRY
RGDV_Ns_Pns10_Rice gall dwarf virus     EKL-PDASGGTLLNQK--SKEQMRVIFSNAKVRIANSINLYVTNNTNSYNEYVREVAEYV
RGDV_Rice gall dwarf virus              EKL-PDASGGTLLNQK--SKEQMRVIFSNAKVRIANSINLYVTNNTNSYNEYVREVAEYV
VP10_RGDV_Ns_Pns10                      EKL-PDASGGTLLNQK--SKEQMRVIFSNAKVRIANSINLYVTTHTNSYNEYVREVAEYV
                                          :            .   .  :    .    :  *     . ::.    :    :    .

GWSS-V2                                 NQEW-DI--GQPTPNLLPVTSTTRD-----------------------------------
RDV_Ns_S9_Rice_dwarf_virus              TQDW-DV--DGGVPATLPQPDTTDDESPVAKPGSSTPTVSKGVDQPEDEEMIRAKVETSK
RDVA_Ns_Pns9_VP9                        TQDW-DV--DGGVPAALPQPDTTDDENPVANPGPSAPTVSKGADQPEDEEMIRKKVETSK
RDV_VP9_Ns_Pns9                         TQDW-DV--DGGVPAALPQPDTTDDESPVTKPGASAPTVSKGADQPEDEEIIHKKVDASK
RDV_Ns_Rice dwarf_virus                 IQDW-DV--DGGVPAALPQPDTTDDERPVTKPGPSTPTVSKGVDEPEDEEMIRKKVETSK
RDVO_VP90_Ns_Pns9                       TQDW-DV--DGGVPAALPQPDITDDESPVTNPGPSAPPVSKGADQPEDEEMIRKKVETSK
WTVNJ_Structural_protein_P9             RTIWADVISDGDVAE-----------------------------------------ETST
WTV_Structural_protein_P9               RTIWADVISDGDVVE-----------------------------------------ETST
RGDV_Ns_Pns10_Rice gall dwarf virus     ADLW-NI--QATTNTQGHENELAAEDFGVL------------------------------
RGDV_Rice gall dwarf virus              ADLW-NI--QATTNTQGHENELADEDFGVL------------------------------
VP10_RGDV_Ns_Pns10                      ADLW-NI--QTTTNTQGHENELAAEDFGVL------------------------------
                                           *  ..
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Fig. 2. Amino acid alignment showing regions with identical sequence (shading and asterisk), for GWSS-V2 Phytoreovirus-like sequence. Legend table provides accession numbers and amino acid sequence lengths. Alignment used T-Coffee© version 1.41 (Notredame et al. 2000).

Fig. 3. Distribution of 3 phytoreovirus host plants in California, Madagascar periwinkle (*Catharanthus roseus*), New Zealand Spinach (*Tetragonia tretragoniodes*), and Cardinal flower (*Lobelia cardinalis*), occurrences shown in shading.

crops, where the GWSS occurs may prove important in the management of these and other emerging agricultural plant diseases.

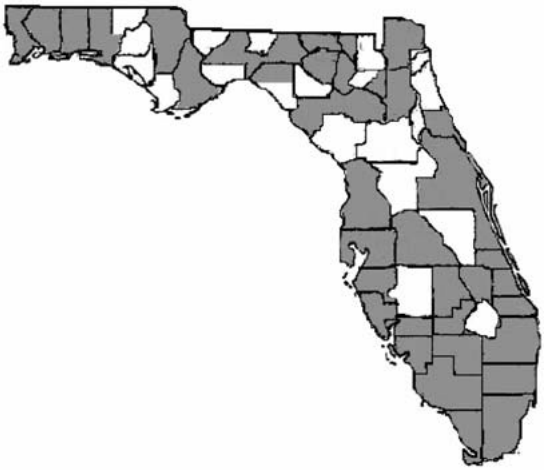

Fig. 4. Distribution of 3 phytoreovirus host plants in Florida, Madagascar periwinkle (*Catharanthus roseus*), New Zealand Spinach (*Tetragonia tretragoniodes*), and Cardinal flower (*Lobelia cardinalis*), occurrences shown in shading.

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