Comparative Analyses of the Coat Protein of Newly Discovered Ampelovirus Using Computer-Assisted Phylogenetic Reconstruction and Analysis: (An Approach for Classification and Characterization)

Rائد الكوني
الجامعة العربية الأمريكية جنين, ralkowni@aauj.edu

Follow this and additional works at: https://digitalcommons.aaru.edu.jo/hujr_a

Part of the Life Sciences Commons

Recommended Citation
Available at: https://digitalcommons.aaru.edu.jo/hujr_a/vol2/iss2/3

This Article is brought to you for free and open access by Arab Journals Platform. It has been accepted for inclusion in Hebron University Research Journal-A (Natural Sciences) - مجله جامعة الخليل للبحوث-أ (العلوم الطبيعية) by an authorized editor. The journal is hosted on Digital Commons, an Elsevier platform. For more information, please contact rakan@aaru.edu.jo, marah@aaru.edu.jo, u.murad@aaru.edu.jo.
Comparative Analyses of the Coat Protein of Newly Discovered Ampelovirus Using Computer-Assisted Phylogenetic Reconstruction and Analysis: (An Approach for Classification and Characterization)

*Raed Alkowni

Arab American University, Jenin - Palestine

Abstract:

The coat protein of the newly found closteroviruses Grapevine leafroll associated virus 9 (GLRaV-9) was used for comparative analysis with other members of the family Closteroviridae. The amino acid alignments using ClustalW demonstrated a significant homology between the GLRaV-9 sequence and the Grapevine leafroll associated virus 5 (86%) and Pineapple mealybug wilt-associated virus 1 (56%). GLRaV-9 and GLRaV-5 were proved to be serologically distinct by using GCG sequence analysis software package that indicate the significant variation (26%) within their polypeptide sequences at the N-terminus of their coat protein. The phylogenetic analysis, grouped GLRaV-9 with mealybug transmitted viruses (GLRaV-1, -3, -5, and PMWaV-1, -2), suggesting the mode of the transmission of this newly found closterovirus.

 الملخص:

البروتين المغلف للفيروس المكتشف حديثا والسمي (9) (Grapevine leafroll associated virus) (Closteroviridae) استخدمت بدراسة تحليلية على أساس المقارنة مع أفراد من العائلة (GLRaV-9) و (Pineapple mealybug wilt-associated virus-1) أو الفيروسا (GLRaV-5) أظهرت تماثل ملحوظ بين المتتالية للفيروس (ClustalW) بنسبة (86%) وكذلك (Pineapple mealybug wilt-associated virus-1) بنسبة (56%). لقد تم إثبات أن الفيروسا (GLRaV-9) و (GLRaV-5) هما مثاليًا غير ذي صلة وذلك باستخدام رزمة برامج للعقل الإلكتروني لتحليل المتتالية والتي تشير إلى اختلاف ذو معنى (26%) للاقتصادية الأمامية للفيروس الذي تم استخدامه في الدراسة وتجمع مع الفيروسات المغلفة (GLRaV-1, -3, -5, and PMWaV-1, -2) المغلفة بوساطة حشرة البكة المغيرة (2). النتائج وجد أن الفيروس الحديث حديث النتائج يشير إلى كيفية هذا الفيروس المكتشف بالفترة اللاحقة.

*Corresponding author: ralkowni@aauj.edu
Introduction:

Closteroviruses are among grapevine infected pathogens that have been isolated and detected from vines showing different types of symptoms, leaf rolling was the mostly observed. Closteroviridae family comprises the longest filamentous stranded RNA plant viruses (Dolja et al., 1994) and the most economically important ones. All closteroviruses isolated from grapevine (but not GRSLV (Zhang et al., 1998)) are associated with leafroll disease complex. Up to now 9 closteroviruses had been isolated from the grapevine and associated to leafroll disease named as Grapevine leafroll associated virus –1 to -9. These viruses induce leaf symptoms range from yellowish (Figure No.1) to reddening (Golino et al., 2002), reductions in yield product as well as influencing the quality of grapes (Woodram et al., 1984).

Closteroviridae compromises three genera: (i) Clostervirus: type species Beet yellows virus; (ii) Ampelovirus: type species Grapevine leafroll virus 3; and (iii) Crinivirus: type species Lettuce infectious yellows virus (Martelli et al., 2002). Biological assays are frequently used for description, but not for distinguishing, of these viruses. Serological assays by production of poly- and/or monoclonal antibodies against the coat protein of these viruses enabled the researcher to distinguish among of them, but low titers in plant tissues or inability to purify some of these viruses are problematic.

Figure 1. Grapevine leafroll disease symptoms observed on different vines. (a) Yellowish symptoms observed on the vine infected with GLRaV-9. (b) Leafroll disease symptoms affecting vineyards and ranging from yellowish to reddening leaves.
still one of the obstacles facing the production of their antisera. Bioinformatic analysis nowadays, using computer programs, make inferences from the data archives of modern molecular biology, and connections among them quite possible, which derive also very useful and interesting predictions (Lesk, 2002). Analysis of these genomes has demonstrated a great deal of diversity in gene content and organization within the family Closteroviridae. Closterovirus genome have at least 7 ORFs called a “minimal set”, two coding for the replication associated with ORF1a (Methyltransferase (MTR), helicase (HEL)) and ORF1b (RNA-dependent RNA polymerase (RdRp) (Klaassen et al., 1996), and other 5 ORFs representing a closterovirus-specific gene block that includes HSP70, and two structural proteins, CP and CPd representing closterovirus-specific gene block required for normal movement of the virus, infection through the plant, and virus assembly (Peremyslov et al., 2000.).

The available sequencing data for these viruses (Martelli et al., 1997; Agranovsky et al., 1994; Karasev et al., 1995; Klaassen et al., 1995; Jelkmann et al., 1997; Fazeli & Rezaian, 2000; Zhu et al., 1998; Ling et al., 1998) make it possible to design carefully species-specific primers for routine diagnosis. Primers specific to grapevine leafroll associated virus 9 had been developed and used for detecting this virus in different universal labs and it was found in some of the major grapevine varieties grown in Australia (Habili and Rowhani, 2002) as well as in some Californian vineyards (Alkowni et al., 2004).

Grapevine leafroll associated virus 9, a new member of the family Closteroviridae, was isolated from grapevine showing mild leafroll symptoms, and subsequently, the full length HSP70 sequence was determined (Alkowni et al., 2004). The full length sequence of this virus is now undergoing research.

In this study we report the complete nucleotide sequence of coat protein of this newly discovered Ampelovirus (GLRaV-9) with comparative analyses of to other different grapevine viruses belonging to the Closteroviridae family by using the available genetic information and computer-assisted phylogenetic reconstructions and analysis. This comparison will be used to make better understanding, characterization and suggestion for classification of GLRaV-9 based on the knowledge of its coat protein comparing with other coat proteins of viruses belonging to the same family.

Materials and methods:

The coat protein sequences and virus-database collections:

The coat protein sequence of the virus GLRaV-9 was obtained from sequenced clones derived from the cDNA library constructed on the virus dsRNA isolated from Helena variety infected with Grapevine leafroll associated virus 9 (Alkowni & Rowhani, 2003). The overlapping clones containing the virus coat protein have been sequenced by using the ABI PRISM® 3100 Capillary Electrophoresis Genetic Analyzer (Ap-
plied Biosystems and Hitachi, Ltd.), an automated sequencing facility (DBS) at the University of California, Davis. The obtained sequence data were processed by Chromas program (Technelysium Pty. Ltd.) and compared. Amino acid sequences of polypeptide of grapevine leafroll associated virus’s coat proteins have been collected from the published NCBI databank (SWISS-PROT database). The accession number of each virus from which their amino acid sequences data collected and used for genomic analysis and phylogentic tree construction, are listed as follows: Grapevine leafroll associated virus 1 (GLRaV-1) (Accession number: AF195822); Grapevine leafroll associated virus 2 (GLRaV-2) (Accession number: AF039204); Grapevine leafroll associated virus 3 (GLRaV-3) (Accession number: AF037268); Grapevine leafroll associated virus 5 (GLRaV-5) (Accession number: AF233934); Beet yellows virus (BYV) (Accession number: BY51931); Lettuce infectious yellows virus (LIYV) (Accession number: NC_003618); Citrus tristeza virus (CTV) (Accession number: CT16304); Little cherry virus 1 (LChV-1) (Accession number: Y10237) Pineapple mealy bug associated virus 1 (PMWV-1) (Accession number: AF414119) and Pineapple mealy bug associated virus 2 (PMWV-2) (Accession number: AF283103). Artichoke mottled crinkle virus (AMCV) coat protein (Accession number: NC_001339) which belong to the family Tombusviridae was used as an outgroup for phylogenetic analysis.

Sequencing and computer-assisted nucleotide and amino acid sequence analysis:
Database searching of similarity to: any amino acid sequences found in that database were conducted by BLAST search programs of the National Center for Biotechnology Information (NCBI). The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families (Altschul et al., 1997). The amino acid sequences of other closteroviruses and phylogenetic outgroups were obtained through the Entrez program at the NCBI and compared to other closterovirus sequences available in its databank. The predicted molecular mass of the polyprotein of virus coat protein was determined by the DNASIS Max program package (Hitachi software Engineering Co., Ltd., UK). ClustalW, a general-purpose multiple alignment programs for DNA or protein sequences was used to create a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. This program was used for alignment of amino acid sequences of polypeptide of GLRaV-9 CP, with the corresponding proteins of other closteroviruses. The hydropath and similarity plotting were done using the web-based Genetics Computer Group (GCG) sequence analysis software package SeqWeb 2.1 (University of Wisconsin, Madison, WI, USA). Phylogenetic tree showing the relationships between the species
and genera of the family Closteroviridae based on the sequence of the CP gene. The neighbour-joining tree was produced by CLUSTAL W (Higgins et al., 1996), which guides the alignment of sequences. To assess more accurately the relationships among closteroviruses, phylogenetic analysis were done with the assistance of the phylogenetic analysis using parsimony (PAUP*4.0) programs (Sinauer Associates, Inc., USA) and bootstrap was used to obtain a consensus tree for a better assessment of phylogenetic relationships.

Results:

Nucleotide sequences of the overlapping clone of Grapevine leafroll associated virus 9 were collected from the automated sequencing facility (DBS) at the University of California, Davis and processed by Chromas program (Technology Pty. Ltd.) and translated to give a total number of 268 amino acid sequence (Figure No. 2).

Similarity searches against protein-protein database searching tool (BLASTP) of the National Center for Biotechnology Information (NCBI), demonstrated a significant homology (P<e-50) between the GLRaV-9 coat protein sequence and the coat proteins of Grapevine leafroll associated virus 5 (GLRaV-5) and Pineapple mealy bug associated virus 1 (PMWV-1) both are belonging to the family Closteroviridae and identified species within the genus Ampelovirus. This lead the suggestion of that GLRaV-9 is indeed belonging to the genus Ampelovirus and thus its mode of transmission could be predicted. In fact the trials to find transmissibility of this virus by mealybug are encouraging.

The predicted molecular mass of GLRaV-9 coat protein was determined by the DNASIS Max program package to give molecular weight of 29465.69 (~29.5KDa) closely similar to GLRaV-5 which has molecular weight of 29317.28. The slight variations among these two virus’s coat protein are due to the variation of their amino acid contents were graphically illustrated (see Figure No.3).

Comparative analysis of GLRaV-9 to those closely related viruses (GLRaV-9, PMWaV-1, and GLRaV-5) were carried out using the multiple alignment program (ClustalW) for protein sequences to show homology between GLRaV-9 coat protein with GLRaV-
Figure 3. Comparison among the amino acid contents of GLRaV-5 and GLRaV-9 showing slight variation among these two virus’s polypeptide in certain amino acids as Serine, ..etc, where it is almost the same in such Cysteine, ..etc.

5 and PMWaV-1 as 86% and 52% respectively. The conserved amino acids among all of them are shown in Figure No.4.

It is clear that both GLRaV-9 and GLRaV-5 are similar in their coat protein (Fig. 4), although they were found serologically distinct by ELISA and western blot analysis (Alkowni et al., 2004). Plot similarity analysis using the SeqWeb 2.1 program provided by web-based Genetics Computer Group (GCG) sequence analysis software package (University of Wisconsin,
The comparison for best fit alignment of the N-terminus half of the coat protein gene sequences of GLRaV-9; compared with same region of the amino acid sequences of the coat protein of GLRaV-5, showed that it had 74% identity while the C-terminus half portion was close to identity. This suggests why both viruses are serologically differing while they are relatively close. Most thought that the first halves of the coat protein amino acids are forming the outer surface of the virus coat protein subunits. This prediction is also proved by Plot structure analysis and using the same GCG sequence analysis software package where it was showed clearly that the first amino acids in the coat protein polypeptide structure having the high hydrophilicity comparing to the rest of their amino acids (Figure No. 6).

Figure 5. GLRaV-9 and GLRaV-5 “Similarity Plot Analysis” using the SeqWeb 2.1 program provided by web-based Genetics Computer Group (GCG) sequence analysis software package (University of Wisconsin, Madison, WI, USA), showing the homology variation among the first half of their polypeptides while they seem to be identical once they are close to C-terminus.
GLRaV-9 coat protein analysis showing that this virus is one of the species belonging to the family Closteroviridae, is also clustering with members of the genus Ampelovirus (GLRaV-5 and PWaV-1). Similarity plot analysis reveals the high similarity with GLRaV-5 showing significant variation within their N-terminus of their polypeptide which predicted to be responsible for enhancing the immunogenecity. Those explain why both viruses are serologically distinct. These analyses support the suggestion of the use of bioinformatics analysis for grouping, classification and characterization of the virus comparing with closely related viruses. Although closteroviruses demonstrate an unusual genetic diversity within the family, they have always been difficult viruses to classify due to their character as phloem-limited, and inadequate established criteria to define closteroviruses thus phylogenies can be used to address this intrafamily evolution (Karashev, 2000). In this study we used the genetic information of GLRaV-9 coat protein as well as other sequences of 10 different closteroviruses and Artichoke mottled crinkle virus (AMCV) coat protein (Accession number: NC_001339) as an outgroup to generate a phylogenetic tree using the using parsimony (PAUP*4.0) programs (Sinauer Associates, Inc., USA) with bootstrap to obtain a consensus tree for a better assessment of phylogenetic relationships among of them. Phylogenetic analyses of coat protein genes have been used to determine the relationships of different closteroviruses (Mount, 2001). Based on phylogenetic analyses of coat proteins...
protein sequences, the 10 virus species were clustered into three major clades (Figure No. 7). While LIYV was grouped alone in clad I, GLRaV-1, -3, -5, -9 and -PMWaV-1, -2 were grouped in clad II. The rest were grouped in clad II, meanwhile AMCV was rooting the tree since it is an outgroup. In fact each clad represents a group of viruses some previously classified based on their mode of transmission. Clade II including mealybug transmitted viruses (Martelli, 2002), while clad I; include the whiteflies transmitted viruses and clad III is for aphid transmitted ones. By using phylogenetic analysis the mode of transmitting of this virus was predicted. This comparative analysis using bio-computing assisted software’s are highly recommended for guiding researcher in classification and characterization of newly discovered viruses.

Figure 7. The relationship of GLRaV-9 coat protein to other 10 different closterovirus species was determined by a phylogenetic tree constructed with bootstrap based on coat protein amino acid sequences. The amino acid sequence of *Artichoke mottled crinkle virus* (AMCV) coat protein was used as the out group. The numbers above the branch lines are bootstrap confidence value. Three major clades: I, II and III, grouping GLRaV-9 with those mealybug transmitted viruses.
Acknowledgement:

The major part of this work was carried out at the Dep. of Plant Pathology, University of California, Davis CA 95616, USA through out my postdoctoral research study (2001-2003)

References :


