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Original Article

Genetic Diversity of Coat Protein Gene of Peanut Stunt Virus (PSV); Its Evolution Governed by Mutation and Natural Selection

Pourrahim R[1*], Rezapanah MR[2, 3], Farzadfar S[1]

1. Plant Virus Research Department, Iranian Research Institute of Plant Protection (IRIPP), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
2. Insect Molecular Virology laboratory, BioControl Department, Iranian Research Institute of Plant Protection (IRIPP), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
3. Iranian Network for Research in Viral Diseases, Tehran, Iran.

Abstract

Background and Aims: *Peanut stunt virus* is a member of the genus *Cucumovirus* in the family *Bromoviridae*, and causes economic yield losses mostly in legume plants worldwide. The synonymous codon usage patterns, which provide significant information about the evolutionary changes that influenced viral survival rates and fitness have not been reported for PSV.

Materials and Methods: The complete coat protein (CP) gene sequences of 73 PSV isolates worldwide, including Iranian isolates from the GenBank database were used for codon usage bias -CUB analysis. To clarify the genetic diversity of PSV, CP sequences were aligned using CLUSTALX2. Maximum Likelihood (ML) tree was reconstructed using 34 representatives of each group by MEGAX using the K2+G+I method with 1000 Bootstrap replicates. The CodonW 1.4.2 package was used for assessing the nucleotide mixtures at the 3rd codon position (A3, C3, T3, and G3%). The Emboss explorer (http://www.bioinformatics.nl/emboss-explorer/) was used for calculating GC content at the first, second, and third codon positions (GC1s, GC2s, GC3s), where the average of GC1 and GC2s is indicated by GC1,2s. The codon usage data for the different hosts were obtained from the codon usage database (available at https://hive.biochemistry.gwu.edu/review/codon).

Results: Phylogenetic analyses using CP sequences clustered the PSV isolates into two main groups (GI & GII), in which subgroups I to V isolates fell in GI however, a new subgroup VI isolates cluster in GII. High nucleotide diversity in the PSV CP gene of new subgroup VI may indicate the recent expansion of these isolates. The average of codon adaptation index (CAI) analyses shows that the host adaptation was highest for subgroup VI, followed by V, II, III, I, and IV subgroups, respectively. A constant and conserved genomic composition CP coding sequences were inferred by low codon usage bias. Nucleotide composition analysis indicates the frequency of amino acid coded by U/C ended optimal codon. This unequal use of nucleotides composition with parity rule 2 (PR2) and the effective number of codons (ENC) plots indicates that the combination of mutation pressure and natural selection are deriving the codon usage patterns in the CP gene but the role of selection pressure is more important. Principal component analysis (PCA) demonstrated that the majority of PSV isolates from subgroups I to V clustered near the origin, which might be due to these isolates being older with a common origin.

Conclusion: Our findings showed that overall codon usage bias within PSV CP gene sequences is slightly biased. The evolution of PSV perhaps reflects a dynamic process of mutation and natural selection to adapt their codon usage to different environments and hosts. This research makes an essential contribution to the understanding of plant virus evolution and reveals novel information about their evolutionary fitness. This study shows the high nucleotide diversity among CP gene sequences of PSV isolates and proposes a new subgroup VI.

Keywords: PSV, codon usage patterns; mutation pressure; natural selection; host adaptation

Introduction

Several mechanisms are responsible for evolution of viruses; of them, recombination, negative selection, and gene flow are most important (1-3). Understanding the evolution of virus-host interactions is so important, due to rapid evolution through genetic recombination, mutation, the potential of adaption to new or resistant hosts (4, 5), fast adaptation to the different environmental conditions, and mostly lack effective chemical compounds (6). As the virus translation is dependent on the host cellular machinery, the

[*] **Corresponding author:**
Reza Pourrahim,
Email: pourrahim@yahoo.com.

interaction of a virus with a particular host must be studied on the basis of its codon usage bias (CUB). A significant role of CUB in the evolution of viruses was reported (7). The codon usage pattern of viruses indicates the evolutionary changes that allow the viruses to optimize their survival and better adapt toward fitness to the external environment and, most importantly, their host (8). Two major models, (i) natural/translational selection and the (ii) mutational/neutral model, explain the codon usage bias (9, 10). The natural selection model proposes that there is a co-adaptation of synonymous codon usage and the transfer RNA (tRNA) abundance to optimize translational efficiency (11). Thus, the codon usage is adaptive because it enables the efficient use of ribosomes and maximizes the growth rate of fast-growing organisms (12). The mutational model postulates that genetic compositional constraints influence the probability of mutational fixation, and this was found in many RNA viruses (13, 14). The GC content is probably to be determined mostly by genome-wide mutation bias rather than by selective forces acting specifically on coding regions. Unfortunately, the studies on CUB and its role in the evolution of plant viruses are limited (13). The recent advancement in sequencing technologies, allow studying the codon usage behavior of viral diseases (15-18).

It is presumed that viral coat proteins (CP) evolved more rapidly than proteins involved in replication and expression of virus genomes (19), thus providing a strong incentive to study the diversity of viruses based on CP genes.

The family *Bromoviridae* is considered as one of the most important families of plant viruses, infecting a wide range of plants (20). *Peanut stunt virus* (PSV), a member of the genus *Cucumovirus*, was first described in 1966 and has since been reported causing epidemics in legume crops worldwide (21). The broad host range and the large number of aphid vector species make them a serious threat to plant health (22). Aphid species including *Acyrthosiphon pisum*, *Acyrthosiphon kondoi*, *Aphis craccivora*, *Therioaphis trifolii*, are among the main aphid species reported from alfalfa fields in Iran (23, Rezapanah, personal observations).

Isolates and strains of PSV have been differentiated based on host symptomology and serology (21). PSV strains were first divided into two subgroups: I (eastern-E type) and II (western-W type) (24). Then a third subgroup was proposed, represented by strains from China (25), and Mi strain was designated as the type member of the PSV subgroup III (26).

In addition, the PSV isolates from *Robinia pseudoacacia* were considered as subgroup VI (27). Our previous analysis indicates the prevalence of PSV-W isolates in Iran (28). Recently a new subgroup comprising only the Iranian isolates is proposed using phylogenetic analyses of CP gene sequences (29). The genetic variability and population structure of PSV have already been studied (24, 29). However, the synonymous codon usage patterns and selection pressure analysis, which provides significant information about the virus evolution as well as gene expression and function, has not been reported.

In this study, patterns of codon usage bias were investigated using 73 complete CP nucleotide sequences of PSV isolates. These analyses reveal novel information about the evolutionary fitness of PSV.

Methods

Viral isolates and phylogenetic analyses: The complete CP gene sequences of 73 PSV isolates worldwide, including our previously studied (28) and other Iranian isolates from the GenBank database were used for CUB analysis (Table S1, Supplementary Materials).

To clarify the genetic diversity of PSV, CP sequences were aligned using CLUSTALX2. Maximum Likelihood (ML) tree was reconstructed using 34 representatives of each group by MEGAX (30) using the K2+G+I method with 1000 Bootstrap replicates. The codon usage data for the different hosts were obtained from the codon usage database (available at https://hive.biochemistry.gwu.edu/review/codon) (31).

Nucleotide Composition Analysis and Effective Number of Codons (ENc): The overall frequencies of occurrence of nucleotides (A%,

U%, C%, and G%), the nucleotide at the third (wobble) position of synonymous codons (A3%, U3%, C3%, and G3%), G+C at the first (GC1), second (GC2), and third (GC3) positions, and G+C at the first and second positions (GC1,2) were calculated for the CP gene sequence of each PSV isolate using CodonW version 1.4.2 (32). The ENc values are used to measure the extent of CUB of a gene, and ENc values ranging from 20 to 61 often determine the degree of CUB (33). The ENc value of a gene at or below 35 indicates strong CUB, whereas the gene having an ENc value of 61 indicates that all synonymous codons are used equally (33).

ENc-GC3 Plot and Neutrality Plot : An ENc against GC3 plot was used to investigate the influence of mutation or natural selection on CUB of PSV CP gene sequences. An ENc-GC3 plot is drawn using the ENc and the GC3s values. If selection is the main force, the ENc values would lie far lower than the standard curve, however, if the mutation is the main force in shaping CUB, the ENc values would lie on or near the standard curve (33). A neutrality plot (GC12s vs. GC3s values) is used to decrypt the selection and mutation factors associated with codon usage. GC3s indicate the abundance of G+C at the third codon position and GC12s represent the average of GC1 and GC2. Each dot in the plot represents a CP gene of an individual PSV isolate. In neutrality plots, if the correlation between GC12s and GC3s is statistically significant and the slope of the regression line is close to 1 (the points positioned on the diagonal line), then mutation pressure is the key factor behind the CUB. Conversely, a lack of correlation between GC12s and GC3s indicates selection pressure is the key factor on CUB (34).

Principal component analysis (PCA) and Codon Adaptation Index (CAI) analyses: The significant tendency in codon usage variation of the PSV CP gene sequences was examined by PCA analysis, which demonstrated the significant tendency in codon usage variation (35). A plot of the 1st axis and the 2nd axis of the isolated strains according to the phylogroups were drawn. CAI is a quantitative measure that predicts the highest relative adaptation of the viruses to their potential host. CAI values range from 0 to 1 and was calculated using a web server (http: //genomes.urv.es/CAIcal/). The sequences with higher CAIs are considered to be preferred over those with lower CAIs (36).

Results and discussion

Phylogenetic analyses clustered the PSV isolates into two main groups, in which isolates of subgroup I to V fell in one group (GI), however, three isolates from Poland and two isolates from Iran and Japan cluster in a separate group (GII). The highest nucleotide diversity was found among subgroup VI, which may indicate the recent expansion of these isolates. No correlation was detected based on hosts or geographical isolation (Fig. 1).

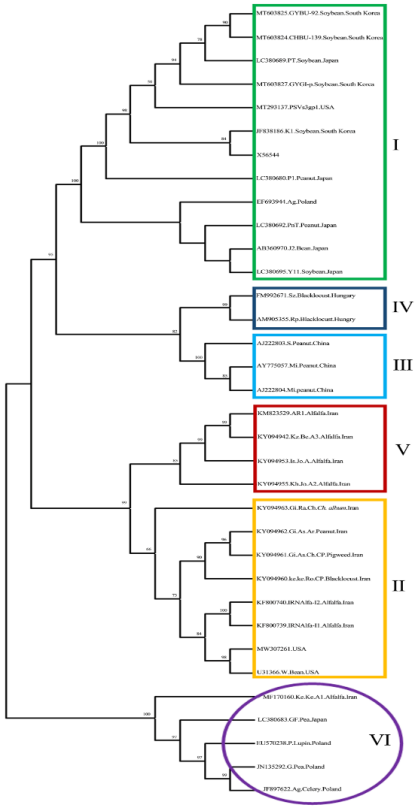

Fig. 1. A ML phylogenetic tree showing the relationship among Peanut stunt virus isolates. The tree was constructed from the full CP sequences of 34 representative isolates. Numbers at each node indicate the percentage of supporting puzzling steps (or bootstrap samples) in the ML method.

Preference of G/U-ended Codon over A/C-ended codon in the AU-rich PSV CP gene: To determine the potential influence of compositional constraints on codon usage, the nucleotide compositions of the PSV coding sequences were determined (Table S1). The mean values of U% (27.08 ± 1.05) and C% (25.97 ± 1.17) were highest, followed by G% (23.48 ± 0.72) and A% (23.44 ± 1.17). The mean values of AU% and GC% were 50.53 ± 1.18 and 49.46 ± 1.17, respectively, whereas the mean values of AU3% and GC3% were 51.35 ± 1.97 and 48.64 ± 1.97, respectively (Table S1). According to the nucleotide occurrence frequencies, PSV CP genes are AU-rich. Therefore, A and U seem to be found more commonly than G and C at the wobble position of CP gene sequences. However, the nucleotides at wobble positions of synonymous codons (A3, U3, G3, and C3) show that the mean values of U3% (34.68 ± 1.97) and C3% (25.19 ± 1.39) were higher than the mean values of G3% (23.45 ± 1.09) and A3% (16.66 ± 2.19). The uneven usage of A3/U3 and G3/C3 nucleotides in the AU-rich CP gene in this study shows that the compositional patterns of the PSV CP gene sequences are more complex than the commonly observed GC- and/or AU-rich compositions of most virus genes. For instance, a GC- or AU-rich genome tends to contain codons preferentially ending with either G/C or A/U. Such trends, when observed, support the influence of mutation pressure. This unequal use of nucleotides indicates the overlapping influences of mutational pressure and natural selection on the codon preferences in the present CP gene sequences.

PSV CP gene shows higher genomic stability and low CUB: The magnitude of CUB of the CP gene of 73 PSV isolates was measured using the effective number of codons (ENc). The ENc values among the present PSV isolates are high and ranged from 53.69 to 58.53 with a mean of 55.39 ± 1.94 (Fig. 2). The higher ENc values in PSV CP gene indicate low CUB, resulting in higher genomic stability. However, the mean ENc values were 53.69, 54.25, 55.29, 56.79, 58.22, and 58.53 for subgroups V, II, IV, I, VI, and III, respectively.

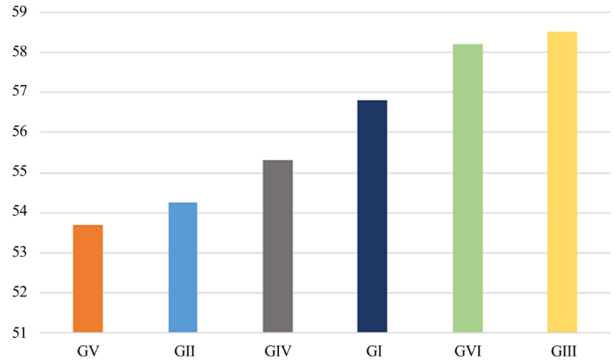

Fig. 2. The ENc values among the present PSV isolates are shown. The mean ENc values were 53.69, 54.25, 55.29, 56.79, 58.22, and 58.53 for subgroups V, II, IV, I, VI, and III, respectively.

The low CUB might be beneficial to PSV on its fitness to the host species with potentially distinct codon preferences. Low CUB was also observed in several RNA viruses, such as Begomoviruses (37), *Rice stripe virus*-RSV (38), and *Potato virus M*-PVM (39), *Papaya ringspot virus*-PRSV (40), *Citrus tristeza virus*-CTV (41), as well.

In an RNA virus population, faster replicators are preferred as the virus shares a common resource with the host for their translational machinery (42). As the RNA-dependent RNA polymerase (RdRP) lacks the 3'–5' proof-reading activity, a high replication rate sometimes decreases the population fitness by introducing deleterious mutations in the viral genome (42). A lower replication rate increases the fidelity, which leads to better fitness of the virus population. Thus, a low CUB of RNA viruses has an advantage for efficient replication in the host cells by reducing the competition between the virus and host in using the synthesis machinery (43).

Mutation pressure and natural selection both play roles in CUB of PSV : The ENc values of PSV isolates ranged from 53.694 to 58.53 at GC3 values of 0.428 –0.526. Most of the PSV isolates grouped below the standard ENc curve, indicating that CUB of the PSV CP gene is influenced by both the mutational pressure and the natural selection (Fig. 3). The role of translation/natural and mutational

selection on CUB in *Papaya ringspot virus* (PRSV) has been also reported (44).

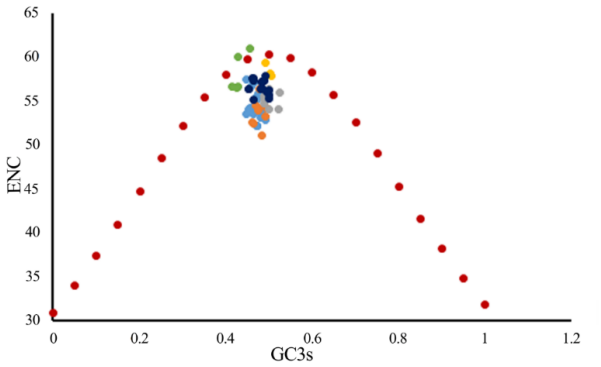

Fig. 3. ENc-GC3 plot analysis of the coat protein (CP) gene sequences of Peanut stunt virus (PSV) isolates. The standard curve plotted while using the codon usage bias (calculated by the GC3s composition only) indicated by red points. Different PSV subgroups are shown with different color markers, GI (dark blue), GII (blue), GIII (yellow), GIV (gray), GV (orange), GVI (green).

Also, it was shown that mutational pressure has a major role in the CUB of plant viruses (45). However, the present study shows that both the natural selection and mutational pressure have an influence on the CUB in PSV, a plant virus, confirming the recent report of Chakraborty et al. (44). In the ENc-GC3 plot, all PSV isolates clustered together, whereas the subgroup VI clustered separately (Fig. 3). This finding indicates differences in the magnitude of natural selection and mutation pressure on CUB among the PSV population.

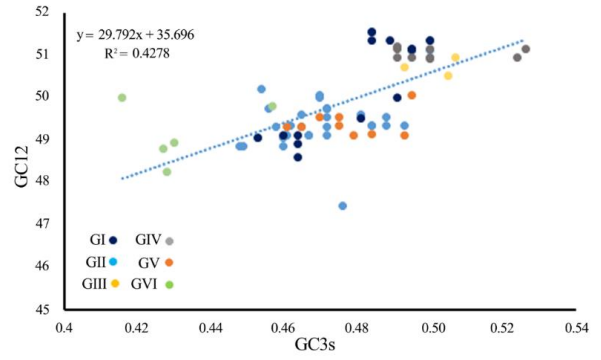

Fig. 4. Neutrality plot analysis (GC12 vs. GC3) for the coat protein gene sequences of PSV isolates. GC12 stands for the average value of GC contents at the first and second positions of the codons (GC1 and GC2), while GC3 refers to the GC contents at the third position of the codons. The blue line is the linear regression of GC12 against GC3. Different PSV subgroups are indicated with different color markers as shown to the left of the figure.

Natural selection plays a key role in shaping the CUB of PSV: The magnitude of mutation pressure and natural selection in CUB was investigated by constructing a neutrality plot (GC12 vs. GC3) (Fig. 4). In the neutrality plot, a positive correlation (r^2 = 0.4278) was indicated between the GC1,2s and GC3s values, which indicates selection pressure is dominant over mutation in shaping codon usage bias of PSV CP gene.

Principal component analysis (PCA) and Codon usage host adaptation: Principal component analysis (PCA), combined with the correlation analysis effectively demonstrated the factors influencing codon usage bias (46). A plot of the 1st axis and the 2nd axis of the isolated strains according to the phylogroups (Fig. 5) were drawn. The PCA analysis demonstrated that the majority of PSV isolates from subgroups I to V clustered near the origin, which might be due to these isolates being older with a common origin.

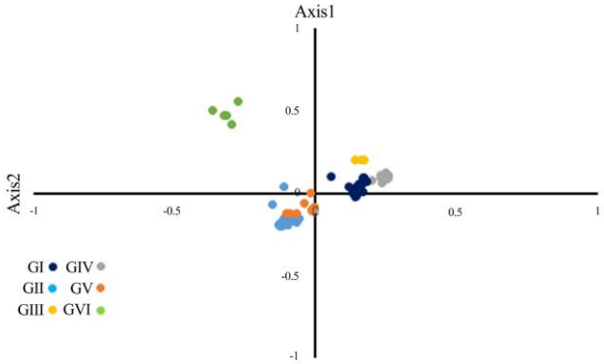

Fig. 5. The AT (A3%/(A3% + T3%)) and GC (G3%/(G3% + C3%)) bias of the CP gene of PSV is shown. Different PSV subgroups are indicated with different color markers as shown to the left of the figure.

The codon adaptation index (CAI) analysis was done for assessment of the codon usage optimization and host adaptation of PSV isolates. The average CAI values of the CP coding sequences were 0.814, 0.766, 0.735, 0.732, 0.700, and 0.690 for subgroups VI, V, II, III, I, and IV, respectively (Fig. 6). These results showed that host adaptation was highest for VI and minimum for IV.

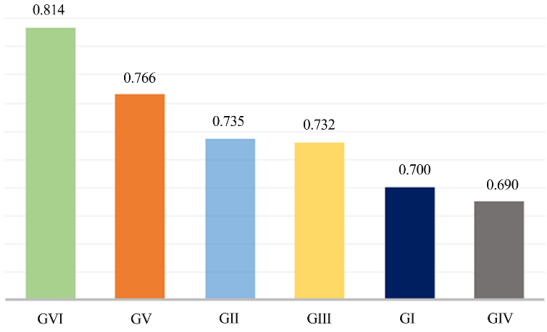

Fig. 6. The average CAI values of the PSV CP coding sequences are shown.

Conclusion

Research on the genetic diversity of viruses provided critical information for understanding virus evolution, geographical origin, virulence variations, and the occurrence of emerging new epidemics. Based on our findings, this study showed that overall codon usage bias within PSV CP gene sequences is slightly biased.

The evolution of PSV perhaps reflects a dynamic process of mutation and natural selection to adapt their codon usage to different environments and hosts. This research makes an essential contribution to the understanding of plant virus evolution and reveals novel information about their evolutionary fitness. In addition, our study shows the high nucleotide diversity among CP gene sequences of PSV isolates and proposes a new subgroup VI.

Acknowledgment

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Conflict of interest

No conflict of interest is declared.

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