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A comparative genomics analysis of maize dwarf mosaic virus and Bermuda grass southern mosaic virus: Distinct N-terminal region of coat protein variations

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Abstract:

Viral population diversity arises from genomic variations, mutations, recombination, and selective pressures, leading to the emergence of distinct viral strains and species. This study focuses on the evolutionary dynamics of potyviruses, particularly the Bermuda grass southern mosaic virus (BgSMV) and its relationship with maize dwarf mosaic virus (MDMV), both members of the genus *Potyvirus*. BgSMV, first identified in Iran, is closely related to MDMV but exhibits key differences, including a 90-nucleotide (NNT) fragment in the coat protein gene and distinct host, vector and temperature preferences. To elucidate the evolutionary mechanisms driving their divergence, we conducted complete genome sequencing, phylogenetic analysis, and recombination detection. Our results indicate that BgSMV and MDMV share a common ancestor but have diverged due to geographical isolation, host adaptation, and ecological factors. Recombination analysis revealed no evidence of genetic exchange between the two viruses, suggesting that their divergence is primarily driven by mutation, deletion, and selection. The study highlights the importance of ecological and host-vector interactions in viral evolution and provides insights into speciation mechanisms within the genus Potyvirus. These findings enhance our understanding of viral population dynamics and the factors influencing the emergence of new viral strains and species.

Keywords: Recombination, Evolution, Phylogenetic analyses, Potyvirus

Introduction

Diversity is a hallmark of life, reflecting the genomic variations that drive population divergence across time and environments (García-Arenal et al., 2001; González et al., 2019; Ruark-Seward et al., 2020). This evolutionary process leads to the emergence of distinct biological entities. Recent advances in molecular analysis have revolutionized our understanding of viral evolution, elucidating the mechanisms of viral change, population dynamics, the emergence of novel strains and diseases, and the complex interactions between viruses, hosts, and vectors. To truly understand these dynamics, it is essential to shift focus from individual viruses to the changes occurring within viral populations over time, examining both drivers and consequences of evolutionary changes (Elena, 2010; Elena et al., 2014; González et al., 2019; Jaag & Nagy, 2010; Ruark-Seward et al., 2020; Schneider & Roossinck, 2001).

Mutations constitute the fundamental substrate of viral evolution. These genomic alterations manifest as: nucleotide substitutions (transitions/transversions), deletions/insertions, recombination events, and genome reassortment. While mutation rates exhibit relative uniformity across viral genomes, differential selective pressures impose non-uniform adaptive constraints, generating distinct evolutionary patterns across genomic regions (Drake & Holland, 1999; Elena et al., 2008; Gibbs et al., 1999; Moya et al., 2000; Schneider & Roossinck, 2001; Valli et al., 2007).

Potyviruses, comprsung the most expansive genus *Potyvirus* within *Potyviridae*, exemplify these evolutionary dynamics. They infect a wide range of monocot and dicot hosts across diverse climatic zones. Transmitted predominantly via aphid vectors in non-persistent mode, potyviruses leverage rapid mutation cycles to overcome host defenses and expand ecological niches (Adams et al., 2005; Adams et al., 2004; Gibbs et al., 2003; Inoue-Nagata et al., 2022; Valli et al., 2015; Wylie et al., 2017).

Potyviruses are characterized by their flexuous, non-enveloped, rod-shaped virions, 680–900 nm in length and 11–15 nm in width. The virions contain a single-stranded, positive-sense RNA genome of approximately 10 kb, encapsidated by roughly 2000 copies of the coat protein (CP). Based on their genome organization and expression strategy, potyviruses are classified within the picorna-like virus supergroup. The RNA genome possesses a VPg (viral protein genome-linked) covalently attached to the 5’ end and a poly(A) tail at the 3’ end. A defining feature of potyviruses is their monopartite genome, containing a long open reading frame (ORF) that encodes a large polyprotein (340–370 kDa). This polyprotein is cleaved by three viral proteases into ten functional proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb (RNA-dependent RNA polymerase), and CP (Adams et al., 2004; Chung et al., 2008; Domier et al., 1987; Gibbs & Oshima, 2010; Urcuqui-Inchima et al., 2001; Valli et al., 2015; Yang et al., 2021). In addition, a +2 frameshift within P3 ORF results in expression of a protein called PIPO (Chung et al., 2008).

Currently, the *Potyvirus* genus includes approximately 200 classified species, organized into various subgroups (Inoue-Nagata et al., 2022). The SCMV subgroup comprises viruses infecting plants within the Poaceae family, particularly those in the Panicoideae subfamily, such as corn, sorghum, and sugarcane (Shukla et al., 1992). These viruses are globally distributed and can cause significant damage to their host plants under favorable conditions, with several species reported in Iran (Masumi & Izadpanah, 2011).

To date, several viruses have been identified within the SCMV subgroup, including *Potyvirus sacchari* (sugarcane mosaic virus, SCMV), *Potyvirus zeananus* (maize dwarf mosaic virus, MDMV), *Potyvirus*

sorghitessellati (sorghum mosaic virus, SrMV), *Potyvirus halepensis* (Johnson grass mosaic virus, JGMV), *Potyvirus zeatessellati* (zea mosaic virus, ZeMV), Iranian Johnson grass mosaic virus (IJMV), *Potyvirus pennisseti* (Pennisetum mosaic virus, PenMV), Bermuda grass southern mosaic virus (*Potyvirus* sp., BgSMV) and Miscanthus sinensis mosaic virus (MsiMV) (Fan, 2004; Fan et al., 2003; Leblanc et al., 2022; Masumi & Izadpanah, 2011; Moradi et al., 2017; Seifers et al., 2000; Shukla et al., 1992). Among these, SCMV, MDMV, IJMV, and BgSMV have been reported in Iran (Izadpanah & Masumi, 2011; Masumi & Izadpanah, 2011; Masumi et al., 2011). Notably, MDMV and BgSMV share significant similarities in nucleotide sequences, serological properties, transmission modes, and host ranges (Biniaz, Izadpanah, et al., 2016; Biniaz, Masumi, et al., 2016; Farahbakhsh et al., 2012; Masumi et al., 2011; Mostafavi Neishaburi et al., 2015; Mostafavi Neishaburi et al., 2025).

Bermuda grass southern mosaic virus (BgSMV) was first identified in 1995 in Jiroft County, Kerman Province, Iran. Subsequent studies have confirmed its widespread distribution in the southern provinces, including Kerman, Bushehr, Hormozgan, Khuzestan, and southern Fars provinces (Masumi & Izadpanah, 1998; Zare et al., 2005). Based on serological, biological, and molecular analyses, BgSMV is recognized as a distinct virus within the *Potyvirus* genus, serologically related to other cereal potyviruses, with the closest relationship to MDMV, followed by SrMV and SCMV (Masumi & Izadpanah, 1998). BgSMV is prevalent in the southern tropical regions of Iran. Phylogenetic analysis of coat protein gene sequences from virus isolates collected in these areas reveals remarkably low diversity, characterized by high nucleotide identity, suggesting the influence of negative selection on the virus evolution (Farahbakhsh et al., 2012).

Despite the similarities between MDMV and BgSMV, they also have key differences . Biologically, BgSMV does not infect Johnson grass and is not transmitted by *Rhopalosiphum maidis*. It also exhibits greater temperature tolerance than MDMV and is primarily found in the warmregions of southern Iran. Genetically, BgSMV contains a 90-nucleotide fragment in the amino-terminal region of its coat protein gene, translating to 30 additional amino acids compared to MDMV(Masumi et al., 2011; Zare et al., 2005). Despite these differences, MDMV and BgSMV display a cross-protective relationship (Zakeri et al., 2012).

Several questions arise from the comparison of MDMV and BgSMV concerning the 90-nucleotide fragment: Is this fragment the result of insertion, recombination, or an ancestral feature lost in MDMV? Did its loss drive speciation? What are the evolutionary implications of this deletion? Finally, are the differences between these viruses solely due to this fragment, or do other genomic regions also play a role? To address these questions, we determined the complete genome of BgSMV and compared it with the genomes of other viruses in the SCMV subgroup, particularly MDMV.

Materials and Methods

Bermuda grass with mosaic symptoms was collected from Bushehr County in November 2016 and transferred to the Plant Virology Research Center (PVRC), Shiraz University. Infection of the plants was verified by Enzyme linked immunosorbent assay (ELISA) using BgSMV antiserum (Masumi & Izadpanah, 2000) and reverse transcription polymerase chain reaction (RT-PCR) using specific primers (Farahbakhsh et al., 2012). The virus was partially purified by differential centrifugation (Farahbakhsh et al., 2012; Masumi & Izadpanah, 2000, 2002; Masumi et al., 2000). RNA was captured using mRNA Capture Kit (Roche) according to

manufacturer guide. Trapped RNA was used as template in RT-PCR.

The full-length cDNA strand was synthesized from viral RNA using reverse (oligo dT) primers and Moloney murine leukemia virus reverse transcriptase (MMuLV-RT, Fermentase, Lithuania). Primary PCR amplification was subsequently carried out using Taq PCR Master Mix (Cinagene, Iran) and 14 pairs of overlapping specific primers designed from the consensus sequence of multiple MDMV isolates or degenerate potyvirus primers (Table 1). (Kalendar et al., 2014). To fill gaps between the contigs, specific primers were designed based on preliminary sequencing data using FastPCR software (Kalendar et al., 2014), followed by RT-PCR with viral RNA as the template. The thermal cycling protocol consisted of an initial denaturation at 94°C for 4 min, 35 cycles of denaturation (94°C for 30 s), annealing (58°C for 45 s), and extension (72°C for 2 min), with a final extension at 72°C for 15 min.

PCR products were inserted into pTZ57R/T plasmid and cloned in *Escherichia coli* DH5a using InsT/A clone PCR product cloning kit (Qiagen) according to the manufacturer's instruction or electroporation method using multiporator system (Hanahan et al., 1991). Recombinant plasmids containing inserts were purified using Plasmid Extraction Kit (Bioneer) and three independent clones for each amplicon were sequenced in both directions (Macrogen Inc., South Korea). Additional primers were designed to internal sequences for subcloning and sequencing (Table 1). The 5′-terminus of the genome was determined using the 5′-specific reverse primer (BgS1-rev, BgS2-rev and BgS3-rev) and the RACE Kit (Roche) (Table 1). The viral sequence data were systematically compared against reference sequences of maize dwarf mosaic virus (MDMV) available in public databases (e.g., NCBI GenBank) to identify conserved and divergent genomic regions. For accurate reconstruction of the complete genome, the BgSMV consensus sequence was assembled from multiple overlapping PCR fragments using DNAMAN 7 (Lynnon Biosoft, Quebec, Canada), leveraging its alignment algorithms and error-correction functions to resolve ambiguities. The final annotated sequence, validated through bidirectional sequencing and manual curation, was deposited in GenBank under accession number KU372146, ensuring its availability for future comparative studies. Due to the sequence similarity between BgSMV and MDMV, primers for BgSMV amplification were designed based on complete MDMV sequences available in GenBank, and these primers were subsequently deposited in the same database. To achieve this, MDMV complete genomes were first aligned using ClustalW to pinpoint regions with >90% identity across isolates. Degenerate primers were then designed based on conserved regions of the sequences using Primer-BLAST software.

Phylogenetic analysis

Multiple sequence alignment with other cereal potyviruses (SCMV, MDMV, PenMV, SrMV, MisMV, JGMV, IJMV) was performed using Clustal X program (version 1.83) software (Thompson et al., 1997). Sequence analysis was performed using MEGA 12 software (Kumar et al., 2024). First, the best DNA/protein model based on maximum likelihood analyses was determined by MEGA 12 software. Phylogenetic analyses based on the model was performed. Phylogenetic trees were constructed using maximum likelihood (ML) method in MEGA 12 software (Kumar et al., 2024). Sequence Demarcation Tool - version 1.2 (SDTv1.2) was used to calculate pairwise nucleotide identity of complete genome and 5'-UTR, 3'-UTR and ten genes of viruses, and polyprotein similarity between sequences (Muhire et al., 2014).

Recombination detection

Recombination events can lead to different evolutionary histories across various regions of a gene,

making it crucial to detect these events before conducting phylogenetic analyses, assessing evolutionary rates, and searching for positive selection.

The initial search for recombination between BgSMV and other potyviruses was performed using the RDP package v.4.101 (Martin et al., 2021) and the SimPlot package (Ray, 1999), employing programs such as Bootscan, RDP, Siscan, and Topal. Recombination signals observed between BgSMV and strains of maize dwarf mosaic virus (MDMV) were subsequently verified.

To ascertain the likelihood of recombination events across the complete genome sequences of all isolates from eight viruses, we utilized SplitsTree5 v 5.0.0_alpha. Furthermore, the RDP4 program (RDP v.4.97) was used to pinpoint potential recombinant and parental sequences as well as to localize possible recombination breakpoints. This analysis involved seven methods: RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan, and 3Seq. A recombination event was considered positive when supported by at least six methods, with a p-value adjusted to 0.05. Phylogenetic incongruence in RDP further validated the potential recombination events.

Further assessment was conducted by reconstructing the phylogenetic tree and a reticulation network from a distance matrix using the T-REX package (Boc et al., 2012). The distance matrix was generated by the TREE-PUZZLE program, employing maximum likelihood analyses with a gamma rate distribution and the NT93 nucleotide substitution model. This matrix was then uploaded to T-REX to construct a hierarchical neighbor-joining phylogenetic tree and network.

To visualize nucleotide similarity among isolates of each species based on the phylogenetic tree, we plotted the similarity over the complete genome using SimPlot software (Ray, 1999). The similarity plot was analyzed using the maximum likelihood method under the F84 nucleotide substitution model, which calculated the variable transition/transversion ratio at each nucleotide site.

Bootscan analysis (Salminen et al., 1995) was performed to determine recombination points through the Bootscan program integrated within SimPlot software. This analysis employed the neighbor-joining method with 100 bootstrap replicates, establishing a 70% bootstrap support threshold as definitive (Hillis & Bull, 1993). A window size of 200 nucleotides, advancing in 20-nucleotide increments, was utilized in both the similarity and boot scanning analyses.

Nucleotide identity

The nucleotide identity of each group of potyvirus species versus other groups, which were determined based on phylogenetic tree, was plotted over the complete genome using SimPlot software (Ray, 1999) to obtain an overview of the situation.

Results

Genome structure

The RNA genome of BgSMV, a monopartite member of the *Potyviridae* family, is 9,589 nucleotides long (excluding the variable-length poly(A) tail) and encodes a single polyprotein. The polyprotein is initiated at nucleotide 140, and terminated by a UGA stop codon at nucleotide 9,355, resulting a 3,071-amino acid residues product. The genome includes 234 nucleotides of non-coding sequences at 3'-region (3'-UTR). A 139 nucleotides 5' untranslated region (5'-UTR) containing multiple CAA repeats that enhance translation (Gallie & Walbot, 1992), and a 3' non-coding region that typically features a poly(A) tail, a hallmark of potyviruses (Biniaz, Masumi, et al., 2016).

The polyprotein is cleaved into ten mature functional proteins by three virus-encoded proteinases. Comparative analysis revealed that all predicted cleavage sites in the BgSMV polyprotein correspond to the same genomic coordinates as those of other potyviruses (Fig. 1 and

Table 2). Noteworthy amino acid substitutions were observed at some cleavage sites in BgSMV compared to MDMV and other cereal potyviruses (Tables 2 and 3). Furthermore, a small open reading frame (ORF) known as PIPO, consisting of 237 nucleotides and coding for 79 amino acids, was identified within the P3 cistron (+2 reading frame). This ORF is conserved throughout the *Potyviridae* family and features a conserved GA6 leading motif (nucleotides 2678 to 2684). The 5' untranslated region (5' -UTR) comprises 139 nucleotides and contains multiple CAA repetitive sequences, which have been reported to enhance translation (Gallie & Walbot, 1992). Overall, most cleavage sites in BgSMV are conserved, with only three amino acid residues differing from the cleavage motifs of MDMV (Table 4).

Phylogenetic analysis

Phylogenetic analysis using maximum likelihood methods on the complete nucleotide sequences, polyprotein, and individual cistrons confirmed that different isolates of the SCMV-subgroup species within the genus *Potyvirus* form distinct clades. BgSMV clusters closely with, but remains separate from, MDMV isolates (Fig. 3). Similar clustering was observed using neighbor-joining methods (data not shown), reinforcing the notion of a more distant relationship between BgSMV and both cocksfoot streak virus and Johnson grass mosaic virus.

Pairwise comparisons of the complete nucleotide and amino acid sequences of BgSMV against eight other cereal potyviruses—including sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), sorghum mosaic virus (SrMV), Johnson grass mosaic virus (JGMV), Iranian Johnson grass mosaic virus (IJMV), Miscanthus sinensis mosaic virus (MsiMV), Pennisetum mosaic virus (PenMV), and cocksfoot streak virus (CSV) demonstrated that BgSMV is most closely related to MDMV. Statistical analyses revealed no significant differences in nucleotide identity between the complete genome and other regions, including the 5'-UTR, 3'-UTR, and polyprotein similarity at $p < 0.05$. The most similar region within the genome was the 5'-UTR, while the P1 gene shows the least similarity. Nevertheless, nucleotide identity variations at each site can effectively differentiate virus species. MDMV has the highest similarity with BgSMV, exhibiting 84.97% nucleotide identity and 91.81% amino acid similarity, while all other viruses exhibit lower similarity (Table 5). Among other potyviruses, BgSMV shared identities of 71.8% (nt) and 77.3% (aa) with SrMV isolates, 69.7% (nt) and 75% (aa) with SCMV isolates, 68.7% (nt) and 71.8% (aa) with PeMV isolates, 68.8% (nt) and 72.2% (aa) with IJMV isolate, 56% (nt) and 50.5% (aa) with JGMV isolates, in the entire genome. The average nucleotide identity among MDMV isolates was 92.675%. These values align with the species demarcation thresholds set by the International Committee on the Taxonomy of Viruses (ICTV).

In contrast, comparisons between the BgSMV genome and other *Potyviridae* species, suggest that BgSMV is distantly related to these viruses. Although the similarities between potyviruses and BgSMV across different genome regions are not statistically significant, the similarity plot indicates that nucleotide similarity is not uniform throughout the genome (Fig. 5). Regions such as the core regions of the P1 and P3 genes, parts of NIa, and the amino-terminal of the coat protein display high variability, while the core regions of the P3 and coat protein genes remain highly conserved (Fig. 5). Despite variability, all genome regions can distinguish between virus species.

Despite prior work by Adams *et al.* (2005) indicating that the CI gene might be the optimal region for distinguishing viruses within this group, the analyses presented here suggest that the coat protein (CP) gene, which includes both variable and conserved regions, offers a more accessible and multifunctional option (Adams et al., 2005). The CP gene plays a crucial role in

virus transmission, pathogenicity, and the generation of symptoms during intracellular and inter-plant movement. This region has been extensively utilized for distinguishing potyviruses (Nigam et al., 2019).For instance, nucleotide similarity between MDMV and BgSMV in the 5' -UTR and 3' -UTR is 93.32% and 90.28%, respectively, indicating high conservation in these regions, whereas the P1 gene shows a lower similarity of 81.51%. Nucleotide identities and amino acid similarities of BgSMV with other viruses are lower than those observed for MDMV. Comparisons of average nucleotide similarity between genes and UTR regions reveal no significant differences when compared to the total genome, indicating that all parts of BgSMV's genome can differentiate it from other species (Table 5).

Phylogenetic analysis based on complete nucleotide and amino acid sequences of MDMV isolates and BgSMV resulted in two main clusters (Fig. 6). The MDMV isolates were grouped into two distinct phylogenetic clusters: American and Eurasian isolates. BgSMV is positioned near the Eurasian isolates, albeit in a separate clade. Nucleotide comparisons highlight that BgSMV has a 90-nucleotide extension at the amino terminus of the CP compared to MDMV, however, certain MDMV isolates from the USA (Missouri and Oklahoma), isolated from maize and Johnson grass, possess a 39-nucleotide stretch in frame (13 amino acids) that exceeds the length of other MDMV isolates (Masumi et al., 2025; Wijayasekara & Ali, 2021).

Recombination Analysis

Recombination analyses were conducted among isolates of SCMV subgroup viruses using various programs within the RDP package. The results indicated no recombination signals between the complete sequence of BgSMV and other viruses. Further investigations using the SimPlot and BootScan programs to analyze recombination between MDMV and BgSMV strains also yielded no evidence of recombination between the two viruses. Similarly, analysis conducted with the RAPR program confirmed the absence of recombination across all 41 isolates of the nine viruses examined in the phylogenetic analysis. Network analysis utilizing the SplitTree program supported these findings (Fig. 7), confirming that recombination events were absent across the isolates.

Based on maximum likelihood methods, initial split tree analyses grouped the haplotypes into seven distinct clades. BgSMV was placed in a separate clade along with the MDMV isolates. In another analysis that included the complete genomes of various MDMV isolates and BgSMV, MDMV isolates were further divided into two groups: American and Eurasian. Notably, two isolates from Ohio, USA (accession numbers JQ403608 and JQ403609), exhibited a deletion of 57 nucleotides (19 amino acids) compared to European isolates, resulting in their classification into a distinct clade.

The network analysis suggests that there were no recombination events, both within and between lineages, as all haplotypes were found to be unique. The phi test yielded a p-value of 1.516E-13, indicating no statistically significant evidence for recombination. This suggests that the observed reticulate patterns may arise from sequence similarities within the population rather than actual recombination events. Importantly, the BgSMV sequence remains independent of these lineages.

Discussion

RNA polymerase errors during viral replication drive mutation, recombination, and variation, especially in RNA viruses, explained by the quasispecies theory (Dolja & Carrington, 1992; Domingo, 2002; Eigen & Biebricher, 1988; Holmes & Moya, 2002; Saitou & Nei, 1987). Advances in next-generation sequencing have improved insights into plant virus phylogenetics, revealing that most virus populations stay relatively stable despite avenues for diversity via mutation and recombination. This stability arises from constraints at the host, vector,

environment, and plant community levels (Acosta-Leal et al., 2011; McLeish et al., 2019), and is strongly shaped by transmission bottlenecks (García-Arenal et al., 2001, 2003; Li & Roossinck, 2004). Viral evolution encompasses divergence and speciation driven by bottlenecks, new host colonization, and environmental changes, which can generate novel strains, however, the mechanisms governing viral fitness and indel events are not yet fully understood.

(Elena, 2010; Elena et al., 2014). . Understanding viral changes requires considering the epidemiological factors, host, vector, and environment, as these influence viral dynamics (Mauck et al., 2012; Ruark-Seward et al., 2020). Studying viruses in wild plants helps reveal their natural diversity and mutualistic relationships with hosts (Roossinck, 2008).

Phylogenetic Analysis of BgSMV and MDMV

Complete genome and polyprotein alignments indicate that Bermuda grass southern mosaic virus (BgSMV) and maize dwarf mosaic virus (MDMV) are the most similar among cereal potyviruses. This finding aligns with previous studies based on coat protein gene sequences (Farahbakhsh et al., 2012; Zare et al., 2005). Phylogenetic analysis reveals that BgSMV is most closely related to MDMV, followed by sorghum mosaic virus (SrMV), sugarcane mosaic virus (SCMV), and others. Notably, MDMV and BgSMV occupy separate branches in the phylogenetic tree.

Ancestral analysis suggests that MDMV, BgSMV, SCMV, and SrMV likely share a common ancestor (Farahbakhsh et al., 2012; Masumi et al., 2012; Zare et al., 2005). A conserved 90-nucleotide (nt) stretch appears to have been present in the most recent common ancestor of these viruses, with variations occurring observed in subsequent generations. Geographical isolation and speciation have resulted in changes to this stretch, which is conserved in BgSMV, deleted in MDMV, and altered in SCMV and SrMV (Rahpeyma Sarvestani et al., 2011). Variations in the N-terminus of the CP are common and have been previously reported for the genus *Potyvirus* (Shukla & Ward, 1988) as well as specifically for members of the SCMV subgroup (Handley et al., 1998; Shukla & Ward, 1988). However, the 90-nucleotides segment in the BgSMV is unique among those reported. The deletion in MDMV's coat protein (CP) gene may have occurred due to new host and geographic conditions, although the precise mechanisms behind these genomic alterations remain unclear.

The conserved DAG motif in the coat protein of potyviruses influences transmission by aphids, located upstream of the 90-nt region in BgSMV (Atreya & Lopez-Moya, 1995; Lopez-Moya et al., 1999; Mostafavi Neishaburi et al., 2025). It is hypothesized that the presence of the 90-nt stretch negatively affects the context of this motif, potentially hindering virus transmission. Future experiments examining aphid-virus protein interactions are necessary to elucidate the mechanisms of virus transmission.

Predictions regarding the secondary structure and positioning of the 90-nt stretch near the DAG motif suggest that its elimination in MDMV does not significantly impact the virus's survival or coat protein function (Nigam et al., 2019; Urcuqui-Inchima et al., 2001). However, the changes in SCMV and SrMV may confer evolutionary advantages. recombination is a significant mechanism of change in viruses, yet analysis using various methods has shown that no recombination has occurred between Bermuda grass southern mosaic virus (BgSMV) and maize dwarf mosaic virus (MDMV). No recombination signals were detected between BgSMV and other potyviruses, particularly in the NNT region of BgSMV. The NNT segment in the N-terminal region of BgSMV does not appear to have entered the virus via recombination. Furthermore, Blast search studies have

not identified any matches for the NNT fragment with other species, and peptide comparisons indicate approximately 70% similarity with the corresponding region in sorghum mosaic virus (SrMV) (Masumi et al., 2025). Given that BgSMV, MDMV, and SrMV share a close common ancestor, it is probable that significant changes occurred in the N-terminal region during the speciation of SrMV, while this region was deleted in MDMV and retained in BgSMV, which has experienced more stable environmental conditions (Masumi et al., 2025).

In the subtropical conditions of southern Iran, the likelihood of recombination between BgSMV and MDMV is low. These viruses have a cross-protection relationship, preventing their coexistence in the same host (Zakeri et al., 2012). Additionally, BgSMV thrives in warmer climates, whereas MDMV prefers temperate and cooler conditions (Biniaz et al., 2017). This cross-protection further diminishes the possibility of simultaneous infection in a single plant, eliminating the chance for recombination between the two viruses. MDMV likely originated in the Mediterranean basin, with Johnson grass as a primary host (Achon et al., 2012; Gell et al., 2010). In contrast, BgSMV, while genetically similar to MDMV, does not infect Johnson grass but thrives in Bermuda grass (*Cynodon dactylon* (L.) Pers.), a widespread perennial grass found in warmer regions. The ecological characteristics of Bermuda grass have fostered stable conditions for BgSMV, reducing its variability through long-term co-evolution, ultimately limiting its host range.

Conclusion

The virus-host-vector-environment relationship forms the epidemiological pyramid, shaped by millennia of coevolution. While each virus has a unique pyramid, general patterns exist across systems (Jeger, 2020). Evolutionary changes in these components can lead to population differentiation and speciation (Fraile & García-Arenal, 2010; Jaag & Nagy, 2010; Lefeuvre et al., 2019). Ecological shifts can trigger new interactions and adaptations, such as viruses switching vectors or hosts, potentially leading to new species through geographic and evolutionary separation (García-Arenal et al., 2001; Roossinck, 1997). These dynamics are vital for understanding disease emergence and management.

In southern Iran, BgSMV maintains stable interactions with Bermuda grass and aphids, preserving its coat protein gene sequence. It can also infect other plants like goosegrass when crops approach infected sources (Masumi & Izadpanah, 2002). Compatibility between viruses and hosts is closely tied to vector adaptability; as vectors adapt, viruses can also infect new hosts, undergo selection, and eventually speciate (Bedhomme et al., 2012; Nigam et al., 2019). Despite their high genetic similarity, BgSMV and MDMV are geographically separated, residing in the Mediterranean basin. The cross-protection relationship between these two viruses reduces their chance of recombination.(Zakeri et al., 2012). While positive selection may play a role in their evolution, geographical isolation and population discontinuity likely enable mechanisms such as Muller's ratchet or mutational meltdown to eliminate interstitial populations (Fraile et al., 1996; García-Arenal et al., 2001; Roossinck, 1997, 2008).

Conflict of Interest

The authors declare no conflicts of interest.

Ethical Considerations

All ethical principles and standards were fully observed in the conduct of this research.

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چکیده

تنوع در جمعیت‌های ویروسی، ناشی از تغییرات ژنومی، جهش، نوترکیبی و فشارهای انتخابی است که به ظهور سویه‌ها و گونه‌های ویروسی منجر می‌شود. این مطالعه به بررسی پویایی تکاملی پوتی‌ویروس‌ها، به‌ویژه رابطه میان ویروس موزائیک جنوبی مرغ (Bermuda grass southern mosaic virus, BgSMV) و ویروس موزائیک کوتولگی ذرت (maize dwarf mosaic virus, MDMV)، پرداخته است. BgSMV که برای نخستین بار در ایران شناسایی شد، علی‌رغم ارتباط نزدیک با MDMV، تفاوت‌های کلیدی از خود نشان می‌دهد. از جمله این تفاوت‌ها، وجود یک قطعه ۹۰ نوکلئوتیدی اضافی در ژن پروتئین پوششی BgSMV و همچنین تفاوت در دامنه میزبانی، انتقال و تحمل دمایی است. مقایسه ژنوم کامل این دو ویروس نشان داد که آن‌ها از یک نیای مشترک نشأت گرفته‌اند و عواملی مانند فشارهای اکولوژیک، سازگاری با میزبان‌های مختلف و جدایی جغرافیایی نقش اصلی در واگرایی تکاملی آن‌ها ایفا کرده‌اند. احتمال وقوع نوترکیبی بین دو ویروس مورد بررسی قرار گرفت، اما هیچ شاهدی از تبادل ژنتیکی بین آنها مشاهده نشد. این یافته حاکی از آن است که واگرایی آن‌ها عمدتاً حاصل فرآیندهای جهش، حذف‌های ژنومی و انتخاب طبیعی بوده و نوترکیبی نقشی در تکامل آن‌ها نداشته است. این پژوهش بر اهمیت عوامل محیطی و رابطه میزبان- ناقل در شکل‌گیری مسیرهای تکاملی ویروس‌ها تأکید می‌کند و بینش‌های جدیدی در مورد سازوکارهای گونه‌زایی در جنس پوتی‌ویروس ارائه می‌دهد. این یافته‌ها به درک عمیق‌تر پویایی جمعیت ویروسی و عوامل مؤثر بر ظهور سویه‌ها و گونه‌های جدید ویروسی کمک می‌کند.

کلیدواژگان: *نوترکیبی، تکامل، واکاوی تبارزائی، پوتی‌ویروس*

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