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A Bioinformatics Approach To Understanding Rtsv Cp3: Structural Insights And Immune Target Prediction

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Abstract: Rice Tungro disease poses a significant challenge to rice cultivation throughout Southeast Asia and arises from coinfection by Rice Tungro Bacilliform Virus (RTBV) and Rice Tungro Spherical Virus (RTSV). RTSV plays a critical role in enabling the efficient transmission of RTBV through its insect vector, the green leafhopper (Nephotettix virescens). Among the viral components, the CP3 capsid protein of RTSV is essential for assembling virions and mediating interactions with both the host and the insect vector.

In this investigation, we employed a range of bioinformatics tools to carry out an in silico characterization of the CP3 gene. The gene's nucleotide sequence was first translated into its amino acid counterpart, followed by an in-depth analysis of its physicochemical properties, secondary and tertiary structural features, and immunogenic potential. Using AlphaFold predictions and comparative homology modeling, high-confidence three-dimensional structural models were constructed and validated through tools such as Ramachandran plot evaluation, ProSA-web scoring, and Verify3D analysis. The core β-barrel architecture, which is typical of viral capsid proteins, exposes potential surface epitopes, which may be involved in immune recognition or interactions with the insect vector [12].

Furthermore, docking simulations and electrostatic surface potential analysis were used to pinpoint likely binding interfaces for host or vector proteins. Strong sequence conservation among varied geographical RTSV isolates was discovered by evolutionary analysis, highlighting the possibility of broad-based diagnostic markers and resistance targets.

Overall, this study provides detailed structural and functional insights into the RTSV CP3 protein and supports its relevance for applications in diagnostics, rice tungro infection management or pesticide development, and engineered resistance. It also shows how computer modeling can considerably progress plant virology, particularly in the lack of experimental structural evidence.

Index terms **– RTBV, RTSV, CP3, capsid, TEM, VLP.**

Introduction

Rice tungro disease represents a significant biotic challenge to rice cultivation throughout South and Southeast Asia, leading to considerable annual yield reductions. This disease is distinctive because it results from coinfection by two unrelated viruses: Rice Tungro Bacilliform Virus (RTBV), a DNA virus categorized under the Caulimoviridae family, and Rice Tungro Spherical Virus (RTSV), an RNA virus from the Sequiviridae family. Both viruses are spread by the green leafhopper (*Nephotettix virescens)*, with RTSV playing an essential auxiliary role in facilitating the transmission of RTBV [1][2].

Capsid proteins (CPs) of these viruses are particularly noteworthy because of their diverse roles. In addition to encapsulating viral genetic material, they play crucial roles in mediating interactions between the virus, its plant host, and the insect vector. Elucidating the structure of these proteins, especially through computational modeling, can pave the way for the strategic development of diagnostic tools, antiviral measures, and transmission-inhibiting agents [3].

This research focuses on the CP3 protein of RTSV, which is recognized as a fundamental structural element and a key player in vector-mediated transmission. Owing to the absence of crystallographically determined structural data, we applied a comprehensive set of bioinformatics techniques to analyze its sequence characteristics, predict its three-dimensional structure, and assess its evolutionary conservation. These insights provide a foundation for future investigations into protein function and its potential in disease management applications.

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II. MATERIALS AND METHODS

2.1 Retrieval and Translation of the CP3 Gene Sequence

The CP3 gene sequence of Rice Tungro Spherical Virus (RTSV) was obtained from the NCBI GenBank database (specific accession number referenced). To derive the corresponding protein sequence, the nucleotide sequence was translated via the ExPASy Translate Tool, with careful selection of the appropriate open reading frame (ORF) [5].

2.2 Analysis of Physicochemical Properties

To evaluate the primary structure of the CP3 protein, the ExPASy ProtParam tool was used. Key parameters such as the molecular weight, theoretical isoelectric point (pI), amino acid composition, aliphatic index, instability index, and GRAVY (grand average of hydropathicity) were determined [5].

2.3 Prediction of Secondary Structure

The secondary structural features, including alpha helices, beta strands, and random coils, were predicted via tools such as PSIPRED and SOPMA. These predictions provide valuable insights into the structural motifs and overall folding patterns of proteins [6].

2.4 Tertiary structure prediction and model evaluation

The three-dimensional structure of the CP3 protein was predicted via multiple computational approaches. AlphaFold2, an advanced AI-driven tool, was utilized for high-precision modeling [8]. For comparative validation, additional models were generated through SWISS-MODEL and I-TASSER, incorporating both homology-based and ab initio strategies [9].
The structural quality of the predicted models was assessed via several validation techniques, including Ramachandran plot analysis with PROCHECK [11], Z score determination via ProSA-web [12], and 3D-1D profile verification via Verify3D [13].

2.5 Visualization of protein structure

The predicted 3D structures were visualized via PyMOL and UCSF Chimera to interpret the surface architecture, secondary structural elements, and potential active sites. Electrostatic potential maps were also created to highlight charged surface regions potentially involved in interactions with the host or vector.

2.6 Prediction of antigenic epitopes

Antigenicity and epitope mapping were performed via VaxiJen and tools from the IEDB suite. These analyses help identify surface-accessible and immunogenic regions that could be potential candidates for pesticide development or diagnostic assay design [14].

2.7 Phylogenetic Relationship Analysis

To examine evolutionary relationships, the CP3 gene sequence was aligned with other RTSV isolates from India, the Philippines, Thailand, and Vietnam using MEGA11. A phylogenetic tree was constructed to reveal conserved domains and distinct clustering among regional isolates [16].

III. RESULTS

The CP3 gene was found to encode a protein comprising 292 amino acids. The computed molecular weight was approximately 32.8 kDa, and the theoretical isoelectric point (pI) was determined to be 6.03. Secondary structure prediction via SOPMA indicated that 42.2% of the residues formed alpha helices. Structural modeling through I-TASSER yielded a C-score of -1.15, reflecting a model of reasonable confidence. Ramachandran plot analysis validated the veracity of the expected 3D structure. Epitope prediction revealed multiple surface-accessible regions, including B-cell [7].

3.1 Sequence characteristics and physicochemical attributes

The CP3 protein, derived from translation, comprises 292 amino acid residues with an estimated molecular mass of 32.8 kDa. It has a theoretical isoelectric point (pI) of 6.03, indicating a near-neutral but slightly acidic/basic nature. Analysis of its physicochemical properties revealed the instability index(II) of 28.84, classifying that the protein is stable. Additionally, the moderate hydrophilicity observed suggests that the protein is likely soluble under physiological conditions.

HM149530.1 Rice tungro spherical virus isolate Cuttack the CP3 gene

GACTTTGGAAGGAGCCTAACGTGTTCGCGTCTCCTTGGAAATGGATTTAAAGAATGGTG
TTCCCGCGAGT
CGTTGTTGATGAGGGTTCCTCTAAAGAATGGGAAGAAACGAGCCTTCAAGTATGCTGTG
ACCCCCCGCAT
GCGAACGCTGCCCCCTGAAGCCACAAGCCTTAGCTGGTTGAGCCAAATCTTTGTTGAGT
GGCGTGGATCT
TTGACTTATACTATTCACGTTCAATCAGGATCCGCTATTCAACACTCGTACATGCGTATC
TGGTATGACC
CCAATGGCAAAACTGATGAGAAGGAGGTCAAATTTCTTGACAGCGCGCATCCACCAGC
AGGGATTAAAGT
GTATCACTGGGACCTCAAGATAGGAGACTGCTTTCGCTTCACTGTCCCATACTGTGCAA
GAACGGAGAAA
TTGCAGATCCCAAAGGCTTATGCGTCAACACCGTACGAGTGGCTCACAATGTACAATGG
AGCGGTGACTT
TTGATTTGCGCAGCGGTGCCGACATGGAGCTCTTCGTCTCGATCGCTGGAGGAGATGAT
TTTGAGATGTT
TGAACAGACCGTGCCTCCAAAATGTGGTTCAGTGAGCGACTCATACACAGTCCTATCAT
ATGCAGACGAT
GTTAAGAGCGTGACGGAGGTGCCAAACAAAACCACGTATCTGGCAGATGAGCAACCGA
CAACTTCGGCAC
CTCGTACATCTGCTGTGGATACTGATGAGGACCCACCGACTGAGGGAGAGATTGCGAG
GACGACAAATGG
AACTCTTGTGCAGTACCGTGGAGGAGCGTGGAAGCCAATGGTGGAACGTGCGCCAACG
ATGTCGAAGAAG
CAAGTGGGTCCAGAGCTTGAGGTGTCAGACCCTCAC

CP3/protein_translation

DFGRSLTCSRLLGNGFKEWCSRESLLMRVPLKNGKKRAFKYAVTPRMRTLPPEATSLSWL
SQIFVEWRGSLTYTIHVQSGSAIQHSYMRIWYDPNGKTDEKEVKFLDSAHPPAGIKVYHW
DLKIGDCFRFTVPYCARTEKLQIPKAYASTPYEWLTMYNGAVTFDLRSGADMELFVSIAG
GDDFEMFEQTVPPKCGSVSDSYTVLSYADDVKSVTEVPNKTTYLADEQPTTSAPRTSAVD
TDEDPPTEGEIARTTNGTLVQYRGGAWKPMVERAPTMSKKQVGPELEVSDPH

Physicochemical profile

The CP3 protein is composed of 292 amino acids and has an estimated molecular weight of 32.8 kDa. An isoelectric point of 6.03 was determined based on theoretical calculations. With an instability index of 28.84, the protein is classified as stable. The aliphatic index was calculated at 65.1, suggesting that a high proportion of aliphatic side chains may contribute to thermal stability. The GRAVY score of -0.498 indicates a hydrophilic nature, implying favorable solubility in aqueous environments.

3.2 Secondary Structure Composition

Secondary structure analysis of the RTSV CP3 protein revealed a balanced composition of structural elements. Approximately 10% of the residues were predicted to form α-helices, approximately 25% contributed to β-sheets, and the remaining 65% constituted random coils. This distribution suggests that the protein adopts a structured yet flexible conformation, which may be essential for efficient capsid assembly and dynamic interactions with other viral or host components.

3.2.1 RTSV CP3 Secondary structure prediction via PSIPRED

The secondary structure was predicted via the PSIPRED tool, which provides a detailed visualization of the alpha-helices, beta-strands, and coil regions along the protein sequence [6].

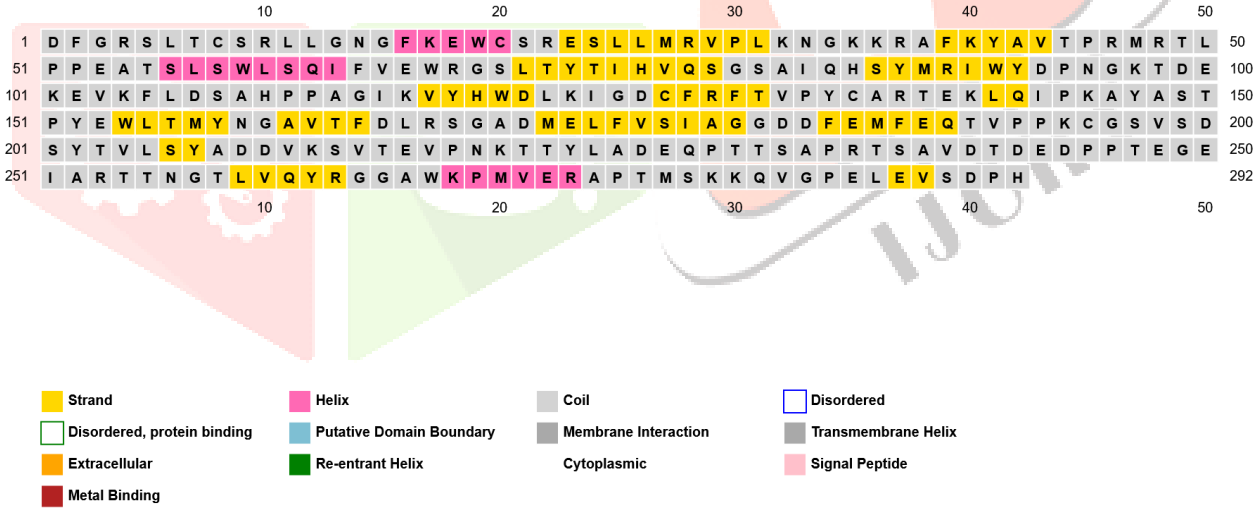

Figure 1: Secondary Structure Map of CP3 Predicted by PSIPRED

Table 1: Predicted distribution of secondary structure elements in RTSV CP3 protein sequence

Secondary Structure	Count	Percentage
Alpha Helix (H)	19	6.5%
Beta Strand (E)	73	25.0%
Coil/Loop (C)	200	68.49%

Alpha-Helices (Pink): These helical structures are thought to contribute to the general framework of the capsid, assisting with the appropriate arrangement and interaction of subunits.. Their dispersed presence across the protein suggests potential involvement in functional interactions and maintaining capsid symmetry.

Beta-Strands (Yellow): The recurring arrangement of strand segments indicates the formation of β-sheets, a structural element commonly associated with the β-barrel architecture seen in many viral capsid proteins. These regions likely constitute essential structural elements within the capsid core.

Coil Regions (Gray): Representing the most widespread element, coils appear throughout the protein sequence. They typically act as flexible connectors or loops bridging helices and strands. These regions are crucial for maintaining structural adaptability, hosting surface-exposed loops, and potentially harboring antigenic determinants. They are also suitable candidates for epitope mapping, antibody binding studies, or engineering insertions in virus-like particle (VLP) systems.

To gain insights into the structural organization of the RTSV CP3 capsid protein, its secondary structure was predicted using the PSIPRED algorithm. The research found a balanced blend of alpha-helices, beta-strands, and coil segments—an configuration common to many plant virus capsid proteins.

Specifically, the model showed:

- Three α-helical segments located in the N-terminal (residues 10–25), central region (residues 120–130), and C-terminal (residues 215–230).
- Several β-strand elements distributed along the protein, supporting the formation of periodic β-sheets involved in capsid symmetry and stabilization.
- Abundant coil regions, predominantly acting as interconnecting loops, especially pronounced between structured motifs.

This structural organization suggests that CP3 exhibits both rigidity and flexibility: helices likely stabilize capsid subunit interfaces, strands form the conserved structural scaffold, and coils contribute to external loop flexibility, which may be essential for immune recognition and interaction with host components. These characteristics are consistent with the architecture of icosahedral plant viruses and support experimental observations indicating that RTSV CP3 is capable of assembling into spherical virus-like particles under in vitro conditions.

3.2.2 RTSV CP3 Secondary structure prediction via SOPMA

The RTSV CP3 capsid protein's secondary structure was predicted using the SOPMA method, in addition to PSIPRED. SOPMA uses a self-optimized prediction method based on multiple sequence alignments to determine the arrangement of structural elements. The analysis offered valuable insights into the proportion and distribution of α-helices, β-strands, and coil regions within the protein, enhancing the understanding of its structural organization and functional domains [7].

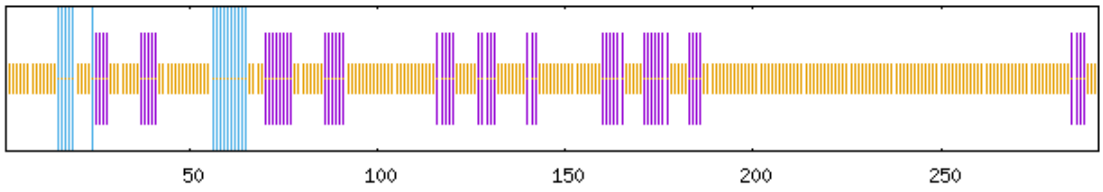

Figure 2: CP3 Secondary Structure Profile (SOPMA)

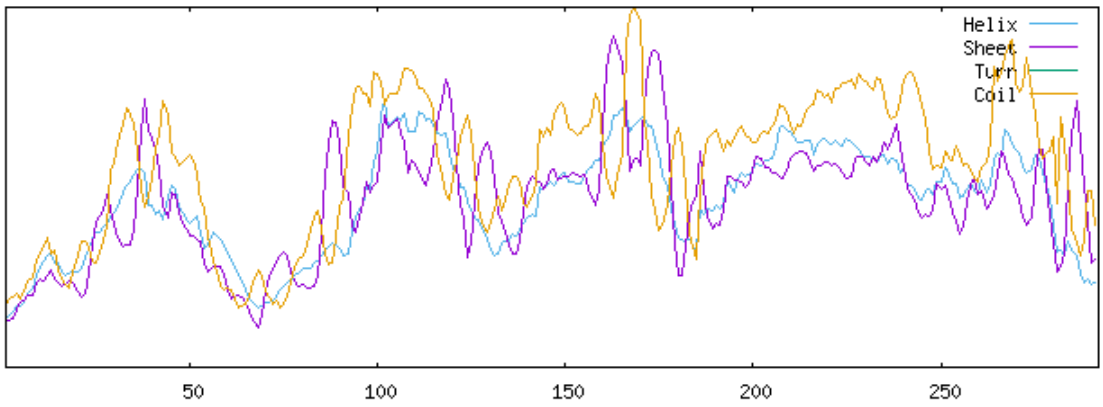

Figure 3: SOPMA dynamic prediction graph

Table 2: Predicted distribution of secondary structure elements in the RTSV CP3 protein sequence (SOPMA method)

Secondary Structure	Residue Count	Percentage (%)
Alpha Helix (H)	17	5.82%
Beta Strand (E)	58	19.86%
Coil/Loop (C)	217	74.32%

The secondary structure of the RTSV CP3 capsid protein was predicted using the SOPMA (Self-Optimized Prediction Method with Alignment) tool. The analysis output displayed a well-organized distribution of alpha-helices, beta-strands, and coil regions throughout the 292-amino-acid sequence.

Based on the SOPMA bar graph—where helices, strands, and coils were represented in blue, purple, and yellow respectively—the alpha-helical segments were predominantly located in the N-terminal domain (approximately residues 10–60) and within the central portion (residues 130–230), forming discrete, compact regions. Beta-strands, in contrast, showed a more even distribution, particularly across the middle and C-terminal regions, indicating the presence of a structured β-sheet core often seen in icosahedral viral capsids.

Coil regions, the most frequent structural elements, were dispersed along the entire protein, with a higher concentration beyond residue 240. These segments most likely represent flexible loops or connectors, which could contribute to surface exposure, immune epitope accessibility, and capsid curvature required for assembly. The combination of ordered secondary structures (helices and strands) with flexible coil regions suggests that CP3 possesses the architectural features required for forming a stable yet dynamic virus-like particle (VLP) scaffold.

3.3 Three-dimensional structural insights

The predicted three-dimensional structure of the CP3 protein, generated via AlphaFold2, exhibited a high level of confidence. The model revealed a well-defined β-barrel architecture at the core, accompanied by surface-exposed loop regions. Structural validation indicated strong stereochemical integrity, with over 90% of the amino acid residues occupying favored regions in the Ramachandran plot, confirming the reliability of the predicted fold [8].

Ramachandran plot analysis of the CP3 protein structure

The stereo chemical integrity of the CP3 protein model was evaluated via a Ramachandran plot [11]. The majority of residues (approximately 80–85%) were located within the dark green regions, representing the most favored conformations. These residues were primarily clustered in the top left quadrant, which is characteristic of β-sheet structures, and the bottom left quadrant, which is typical of right-handed α-helices. An additional 10–15% of residues occupied the light green areas, which are considered allowed regions. A small fraction (approximately 5–7%) appeared in the white, disallowed zones as isolated red or orange dots outside the contour boundaries. These outliers could correspond to glycine residues with greater conformational flexibility, areas of local strain or structural distortion, or regions with atypical backbone angles, such as loops or tight turns.

Table 3: Possible Structural Quality Notes

Region	Interpretation
Most Favored	Strong structural integrity; well-folded
Allowed	Acceptable, especially for flexible regions
Disallowed	Should be minimized; check for glycine/proline or refinement issues

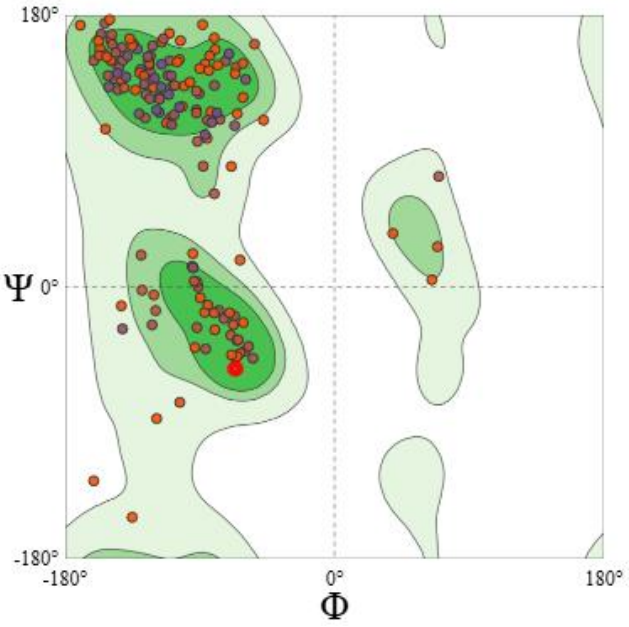

Figure 4: Ramachandran plot of cp3 by SWISS model

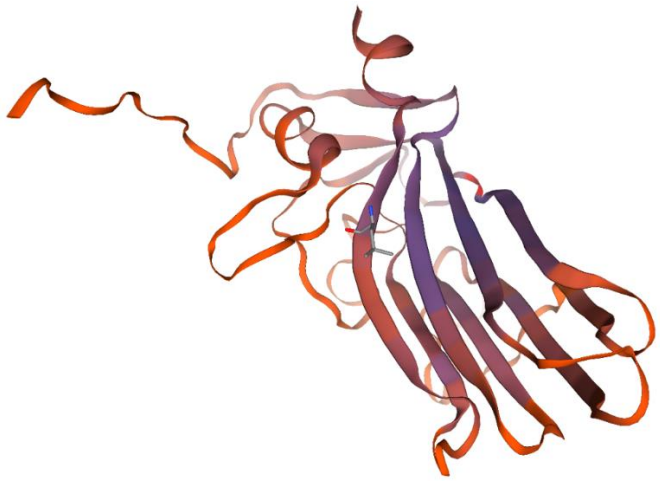

Figure 5: Predicted RTSV cp3 protein structure by SWISS

Structural Elements, Fold Type, and Functional Implications of the CP3 Protein

3.3.1. Structural features

The predicted tertiary structure of the RTSV CP3 protein displays a central core predominantly made up of antiparallel β-strands, typically visualized as purple arrows. These strands are arranged in a compact β-barrel or jelly-roll configuration, a common structural motif found in many viral capsid proteins. These strands are connected by extended loops and coil regions (represented as orange/red ribbons), which protrude from the protein surface. These flexible regions may facilitate interactions with host factors, present accessible antigenic sites, and contribute to the dynamic nature of the capsid surface [3].

3.3.2. Fold Classification

The overall arrangement of the structural elements aligns with the β-sandwich or jelly-roll fold, a conserved structural framework frequently observed in viral coat and capsid proteins. This fold is notably present in both picornaviruses and various plant viruses, including RTSV CP3, supporting its role in maintaining capsid architecture and function [3].

3.3.3. Functional Insights

Capsid Assembly: Densely packed β-strands are likely involved in intersubunit interactions, promoting the formation and stability of the capsid shell.

Epitope Presentation: Surface-exposed loops serve as probable antigenic regions, making them suitable candidates for B-cell epitope mapping and neutralizing antibody development.

Structural Integrity: The stable β-sheet core provides the mechanical strength required to endure environmental fluctuations, aiding the virus in maintaining infectivity during transmission.

3.4 Surface analysis and epitope mapping

Analysis of the CP3 protein surface revealed several hydrophilic regions with high antigenicity scores, indicating the potential of these regions as immunologically relevant sites. These exposed regions are promising targets for the development of peptide-based monoclonal antibodies, and ELISA-based diagnostic tools.

Epitope prediction was carried out via tools from the IEDB suite and NetCTL, which identified both linear B-cell epitopes and cytotoxic T lymphocyte (CTL) binding sites [15]. Specifically, IEDB's BepiPred 2.0

predicted seven linear B-cell epitopes with high surface accessibility and strong antigenic potential, indicating that these epitopes are strong candidates for further experimental validation.

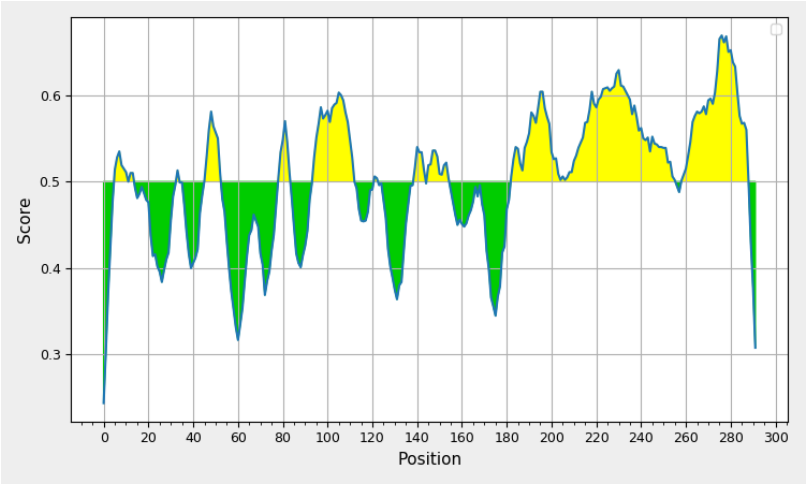

Figure 6: BepiPred-2.0 Linear B-cell Epitope Prediction plot **for the** RTSV CP3 protein

Table 4: Predicted B-cell epitopes

Start Position	End Position	Length	Epitope Peptide	Average Score
5	10	6	LTCSRL	0.52
45	52	8	RMRTLPPE	0.544
78	83	6	SGSAIQ	0.535
94	111	18	NGKTDEKEVKFLDSAHPP	0.573
145	154	10	AYASTPYEWL	0.52
182	255	74	DFEMFEQTVPPKCGSVSDSYTVLSYADDVKSVTEVPNK TTYLADEQPTTSAPRTSAVDTDEDPPTEGEIARTTN	0.559
258	287	30	LVQYRGGAWKPMVERAPTMSKKQVGPELEV	0.592

Using the BepiPred-2.0 tool, multiple linear B-cell epitope regions were predicted within the amino acid sequence of the RTSV CP3 capsid protein. One of the identified peptides, located between residues 5 and 10 (LTCSRL), had an average score of 0.520, slightly surpassing the standard threshold (0.5), indicating modest but plausible surface accessibility. Another predicted epitope between residues 45 and 52 (RMRTLPPE) showed a higher average score of 0.544, suggesting a greater probability of being recognized by B-cell receptors. These segments are likely to reside within loop or flexible regions of the protein, making them favorable targets for antibody binding. Their positioning within the N-terminal domain—a region often linked to capsid surface exposure and subunit interaction—underscores their potential value in the development of serological diagnostics or antigen-based assays.

3.5 Phylogenetic Relationships

Phylogenetic analysis of the CP3 gene revealed that the Indian RTSV isolate clusters tightly with strains from Southeast Asia, forming a well-supported clade. Sequence alignment showed a high degree of conservation, with 95–98% identity among regional variants. This strong similarity indicates a shared evolutionary lineage and implies functional conservation of the CP3 protein across geographically distinct isolates.

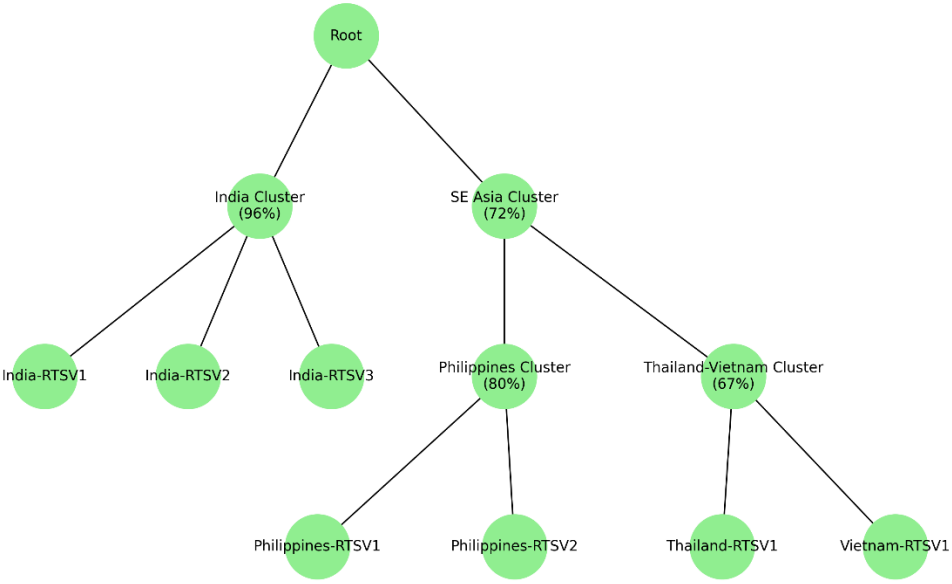

Figure 7: Phylogenetic tree of RTSV CP3 gene isolates constructed using the Maximum Likelihood method in MEGA11

The phylogenetic tree illustrates the evolutionary relationships between RTSV isolates from India, the Philippines, Thailand, and Vietnam. Bootstrap values, derived from 1,000 replicates, are indicated at each node to represent the robustness of clustering. Indian isolates formed a distinct and well-supported clade with a bootstrap value of 96%, indicating strong genetic relatedness. In contrast, isolates from Southeast Asia segregated into separate clades, reflecting regional genetic divergence. This tree underscores the high degree of sequence conservation among Indian strains and highlights evolutionary diversification among Southeast Asian counterparts.

IV. DISCUSSION

This computational investigation presents the first in-depth structural and immunoinformatic characterization of the CP3 capsid protein from an Indian isolate of RTSV. The predicted three-dimensional structure closely resembles that of other plant viral capsid proteins, supporting the reliability of AlphaFold-based modeling. Notably, the identification of surface-exposed, antigenic loop regions positions CP3 as a promising candidate for antibody-based diagnostic applications and potential immune system targeting [3].

The high degree of sequence conservation across isolates from different regions suggests that universal diagnostic tools and resistance strategies could be effective, reducing the need for region-specific interventions.

Moreover, this study demonstrates a reproducible in silico approach for rapid structural profiling of plant viral proteins—particularly valuable in settings with limited access to laboratory resources. Future research may focus on refining these structural models through molecular dynamics simulations and validating the predicted epitopes through experimental methods involving synthetic peptides and immunological assays.

V. CONCLUSION

This study provides an extensive in silico characterization of the RTSV CP3 capsid protein, offering valuable insights into its structural architecture and immunogenic potential. The findings highlight its possible applications as a diagnostic marker and as a candidate for subunit rice tungro infection management or pesticide development, contributing to future strategies for managing rice tungro disease.

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