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In silico Characterization and Homology Modelling of Potato Leaf Roll Virus (PLRV) Coat Protein

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Polerovirus (Family-*Luteoviridae*) are one of the most destructive viruses causing detrimental diseases in vegetable crops in tropical regions of the world including India. Four species viz. potato leaf roll virus (PLRV), potato virus Y(PVY), potato virus X(PVX) and potato virus S(PVS) are known to cause different diseases in potato crops. Of the various viral diseases inflicting potato crops, potato leaf roll disease is the most destructive and widely distributed. They cause huge agro-economical losses (90%) worldwide and thus are the subjects of immense concern. PLRV is a phloem-limited spherical virus transmitted by several aphid species in a persistent manner. A study was performed in order to detect the infection of potato leaf roll virus from different regions of Bihar. These infected samples were diagnosed first using DAS-ELISA for the PLRV infection and later, coat protein was amplified and sequenced from PLRV positive sample. Phylogenetic tree deduced

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based on the nucleotide sequence of the coat protein gene gene showed a distinct divergence of PLRV isolates in two major clades. The molecular weight of the predicted protein sequence of 203 amino acids was found 22617.06 daltons while theoretical pI was 5.22. The extinction coefficient of predicted coat protein was 0.836. An attempt was taken in order to illustrate the 3D model of the coat protein which was further verified using Ramachandran plot. The model structure obtained using Swiss-Model had 92.9% residues in the most favourable region of the Ramachandran plot (Fig. 3c) and showed Z-score for bond angles, chi-1/chi-2 correlation and Ramachandran Z-score were 1.457, 1.773 and -2.633 respectively which exhibited considerably good model quality.

Keywords: Coat protein; potato leaf roll virus; phylogenetic analysis; structural analysis; homology modeling.

1. INTRODUCTION

Potato Leaf Roll Virus (PLRV), a member of genus Polerovirus (Family-*Luteoviridae*), are icosahedral viruses with small (6-kb) RNA genomes that infect phloem-associated tissues of their plant hosts [1]. PLRV cause a major menace for the potato production all over the world [2]. It is transmitted to potatoes solely in a Persistent manner by aphids (*Myzus persicae*) is the most efficient vector [3]. Amino acid residues in a surface oriented loop of the coat protein that are critical for virus assembly and stability, systemic infection of plants, and movement of virus through aphid vectors [4]. Primary symptoms of PLRV appear mainly in young leaves at top of the plant with upright, pale-yellowish, purple or reddish and rolled characters while secondary symptoms are more serious than primary symptoms with stunted growth, rolled and leathery leaves [5]. The genome of PLRV consists of positive sense single stranded RNA and is divided into two parts by a small non-coding RNA and harbors nine open reading frames (ORFs) numbered from 0-8 coding for proteins, P0-P7 and Rap1, respectively. P3, and P4 encode capsid protein (CP) and the movement protein (MP) respectively.The RNA genome of Potato leaf roll virus (PLRV) contains six large open reading frames (ORFs). The 59-located ORFs encode a potential silencing suppressor protein (P0; 6) and RNA polymerase (ORF1 and ORF1/2) (7). Within the 39-located gene cluster, ORF3 encodes the major capsid protein (CP; ~23 kDa), and ORF4, which is contained within the CP gene in a different reading frame, encodes a movement protein (P4; 8).

Nucleotide sequence information of the target genes, especially the level of variability of the virus genes targeted [6-11]. RNAi mediated gene silencing [2,12,13] and CP mediated virus suppression [14-17] are some technologies used against viruses. To develop the resistance against such viruses, coat protein is an important target, so the present study was undertaken to obtain sequence and structural information on coat protein gene of PLRV infecting potato crops. Evolutionary divergence of the virus was predicted and homology modelling was performed to predict the structure of CP.

2. MATERIALS AND METHODS

2.1 Isolation of CP Gene and Analysis of Sequence

Potato crop showing PLRV infected symptoms such as primary symptoms on young leaves as upright rolling and slightly pale in colour and secondary infection as dry and leathery leaves were collected from the different regions of Bihar state like Patna, Nalanda, Kahalgaon, Banka etc. Once the samples were serologically identified through DAS-ELISA, then total RNA was isolated using Thermo Scientific RNA Purification Kit and cDNA was synthesised by the help of Thermo Scientific cDNA Synthesis Kit. On the basis of available sequences of Coat Protein of different isolates of PLRV in Gene Bank, forward (AATGTGGCAACCCAGAAGTG) and reverse primers (GTTAGCGCGCCTGGCAA) were designed. These primers used to amplify the desired sequences of Coat Protein of the positive PLRV sample collected from Patna district. The thermal cycling used for amplification of DNA was 94°C for 5 min (initial denaturation), 35 cycles at 94°C for 45s (denaturation), 49°C for 40s (annealing), 72°C for 45s (extension), and 72°C for 7 min (final extension).The amplified PCR product was then cloned through Thermo Scientific Cloning kit and sequenced and analysed with Basic Local Alignment Search Tool (BLAST).

2.2 Phylogenetic Analysis and Tree Construction

Phylogenetic analysis was conducted using Maximum Composite Likelihood model in MEGA X (Molecular Evolutionary Genetics Analysis Version 10.0) software. The tree was constructed showing the evolutionary divergence of 40 PLRV isolates taken from different parts of the world.

2.3 Protein Prediction and Physico-chemical Analysis of Predicted Protein Sequences

Nucleotide sequence obtained from the sequencing of the Coat Protein was further translated into its amino acid sequence using Expasy-Translate Software. Chemical Composition of the amino acids sequence was analysed by using Prot Pram Software.

2.4 Homology Modelling of Coat-protein and Validation of 3D Model

Alignment was done using the template and a 3D Model was prepared through different softwares such as SWISS Model, Phyre2 and CBS server. The developed models exhibited 80-90% similarity to the available 3D models. The pdb file of the developed model was generated by the help of Rasmol or Jmol software. Finally validation of the 3D structure was done by constructing Ramachandran plot using softwares such as SAVES and What if that shows the allowed regions of different amino acids present in the Coat Protein.

3. RESULTS AND DISCUSSION

3.1 Isolation of CP Gene and Analysis of Sequence

RT-PCR yielded an amplicon of about 650 bp of the isolate collected from Patna district. BLASTn result showed a maximum of 95% sequence identity and E-value zero with the isolate from PLRV (India). Nucleotide sequences of isolates from various regions of India and worldwide with 92-95% sequence identity and E-value zero after BLASTn were selected (Table 1) for phylogenetic analysis. The nucleotide sequence of the coat protein gene was:
GGGTGGCTGTGCAAAACCCCAAATAGGTAGA
CTCCGGATCAGAGCCTGGTCCAAACCCACAA
C
AACACCCTCTCCAACTCCCCAGAAGCACGAG
CGATTCATTGCTTACGTTGGCATACCTATGCT
AACCATTCAGGCTAGGGAGAACGACGACCAA
ATCATATTGGGTTCCTTAGGGAGCCAAAGGA
TGAAATATATAGAGGACGAGAACCAGAACTA
TACAAATGTTAGTTCTGAGTATTACTCTCAAT
CGAGCATGCAAGCCGTCCCTATGTATTACTT
CAATGTCCCGAAAGGGCAATGGTCAGTCGAT
ATCAGGTGAGAAGGGTCTCCCCCCACTAGCA
GCCCCTGAGACACAAAGCGGGGTAGGAGAG
TGGGGATGATCGTGTATTAAAACGCGGTCTC
GGATTTTGGGGATGTTGGTGAAGCGGATGGT
GTCAAAATTTCGAAGCTACGCAACGATAACA
CCTACCGCCAAGGTCACCCAGAACTTGAAAT
CAACTCGTGTCATTTTCGAGAGGGGGAACTC
GTTGCACGGGACGCTACAATTAGCTTCCACG
TTGAAGCGCCTACTGATGGGCGATTCTTTCT
CGTTTGTCACGATATCCAG.

The above sequences were aligned using Mega X software and the completely aligned portion of 627 bp was taken for further study.

3.2 Phylogenetic Analysis and Tree Construction

Phylogenetic tree deduced based on the nucleotide sequence of the CP gene (Fig. 1) showed a distinct divergence of PLRV isolates in two major clades. Clade I included accession from Australia while clade II included remaining. Isolates of India was placed together in sub group with proximity of 0.051. It is far apart from isolate of Australia with a distance 2.366. USA, Canada, Peru, Czech Republic, U.K are some closer isolates with their Phylogenetic distance range from 0.059 to 0.071. All isolates were placed at an average distance of 0.118 with each other.

3.3 Protein Prediction and Physico-chemical Analysis of Predicted Protein Sequences

The nucleotide sequence of coat protein was translated into protein sequences using Expasy-Translate tool. The predicted protein sequence was then taken for the secondary structure prediction using Prot Pram server. The molecular weight of the predicted protein sequences of 203 amino acids was found 22617.06 daltons while theoretical pI was 5.22. In the Secondary structure prediction, the below table shows the different amino acids present in the coat protein gene of PLRV with Physiological and Chemical Parameters (Table 2).

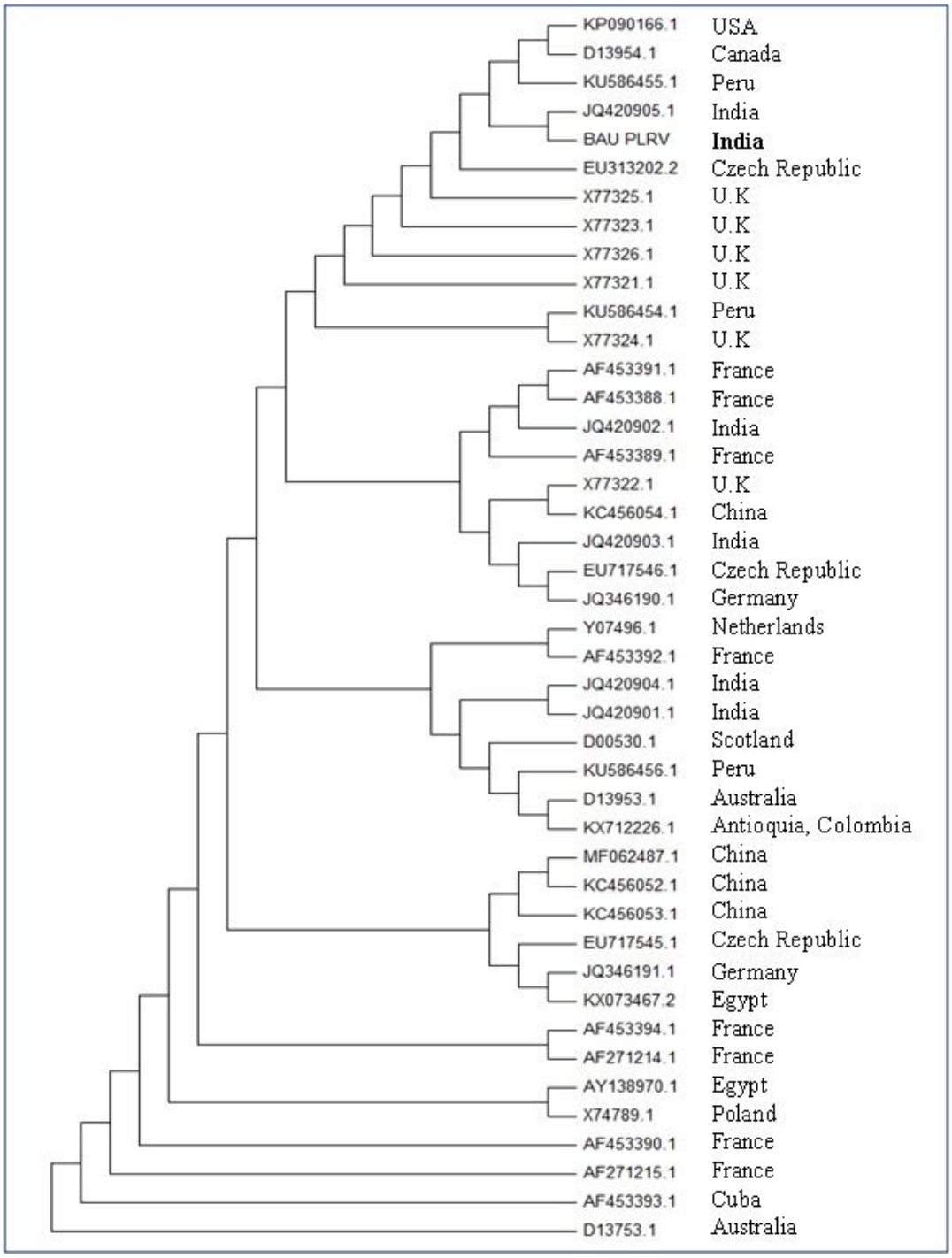


Fig. 1. Phylogenetic analysis of coat protein of PLRV isolates

The extinction coefficient of predicted coat protein was 0.836. The extinction coefficient indicates how much light absorbed by a protein at a certain wavelength. The instability index (42.68) provides an estimate of the stability of protein in a test tube. The putative PLRV coat protein was found to be quite unstable. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains. GRAVY value, which is calculated as the sum of

hydropathy values of all the amino acids divided by the number of residues in the sequence, for the protein under study was -0.667, indicating the possibility of better interaction with water.

Table 1. Accession number, host, region, query cover and sequence identity of PLRV isolates with isolate from BAU (Bhagalpur)

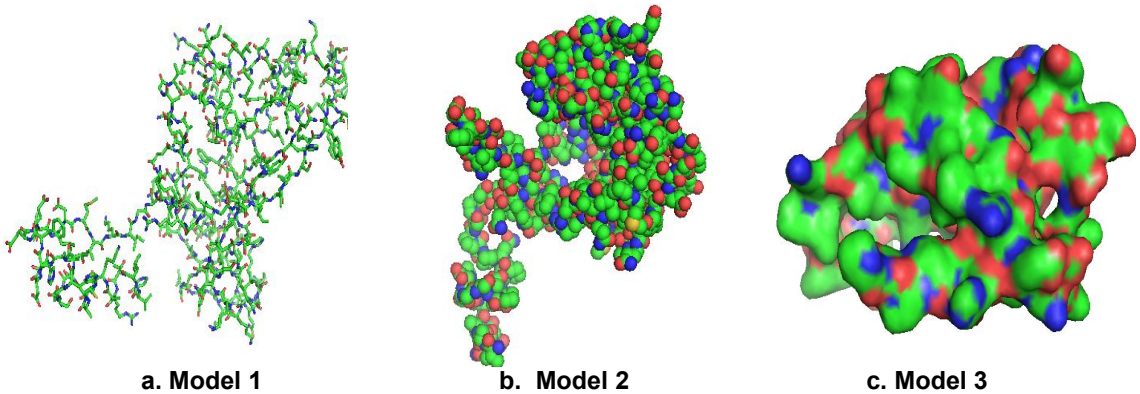
S. No	Accession Number	Host	Place	Query cover	Identity	Phylogenetic distance
1	BAU_PLRV	Potato	India	ref	ref	Ref
2	X77321.1	Potato	U.K	100%	94%	0.059
3	KU586454.1	Potato	Peru	100%	94%	0.063
4	KP090166.1	Potato	USA	100%	93%	0.069
5	D13954.1	Potato	Canada	100%	93%	0.071
6	KU586455.1	Potato	Peru	100%	94%	0.063
7	JQ420905.1	Potato	India	100%	95%	0.051
8	X77326.1	Potato	U.K	100%	94%	0.059
9	X77325.1	Potato	U.K	100%	94%	0.061
10	X77323.1	Potato	U.K	99%	94%	0.059
11	X77324.1	Potato	U.K	100%	94%	0.063
12	X77322.1	Potato	U.K	100%	94%	0.063
13	AY138970.1	Potato	Egypt	100%	93%	0.067
14	X74789.1	Potato	POLAND	100%	93%	0.067
15	EU313202.2	Potato	Czech Republic	100%	94%	0.063
16	EU717546.1	Potato	Czech Republic	100%	94%	0.063
17	JQ346190.1	Potato	Germany	100%	93%	0.071
18	JQ420903.1	Potato	India	100%	94%	0.063
19	KC456054.1	Potato	China	100%	93%	0.071
20	Y07496.1	Potato	Netherlands	100%	93%	0.067
21	AF453392.1	Potato	France	100%	93%	0.071
22	MF062487.1	Potato	China	100%	93%	0.067
23	KC456052.1	Potato	China	100%	93%	0.067
24	KC456053.1	Potato	China	100%	93%	0.069
25	JQ420904.1	Potato	India	100%	93%	0.071
26	JQ420901.1	Potato	India	100%	93%	0.069
27	KU586456.1	Potato	Peru	100%	93%	0.073
28	D00530.1	Potato	Scotland	98%	93%	0.073
29	AF453391.1	Potato	France	100%	93%	0.067
30	AF453388.1	Potato	France	100%	93%	0.067
31	AF453389.1	Potato	France	100%	92%	0.081
32	JQ420902.1	Potato	India	100%	93%	0.067
33	AF453393.1	Potato	Cuba	100%	93%	0.074
34	AF271215.1	Potato	France	100%	93%	0.074
35	JQ346191.1	Potato	Germany	100%	93%	0.065
36	EU717545.1	Potato	Czech Republic	100%	93%	0.069
37	AF453394.1	Potato	France	100%	93%	0.071
38	AF271214.1	Potato	France	100%	92%	0.075
40	AF453390.1	Potato	France	100%	93%	0.069
41	KX073467.2	Potato	Egypt	96%	93%	0.077
42	D13953.1	Potato	Australia	96%	90%	2.366
43	KX712226.1	Potato	Antioquia, Colombia	96%	90%	0.111

Table 2. Physiological and chemical analysis of predicted coat protein of potato leaf roll virus

Physiological and chemical parameters	Value
Number of amino acids	203
Molecular weight	22617.06
Theoretical pI	5.22
Total number of negatively charged residues (Asp + Glu)	26
Total number of positively charged residues (Arg + Lys)	19
Extinction coefficients:	0.836, assuming all Cys residues are reduced
Instability index:	42.68
Aliphatic index:	63.79
Grand average of hydropathicity (GRAVY)	-0.667

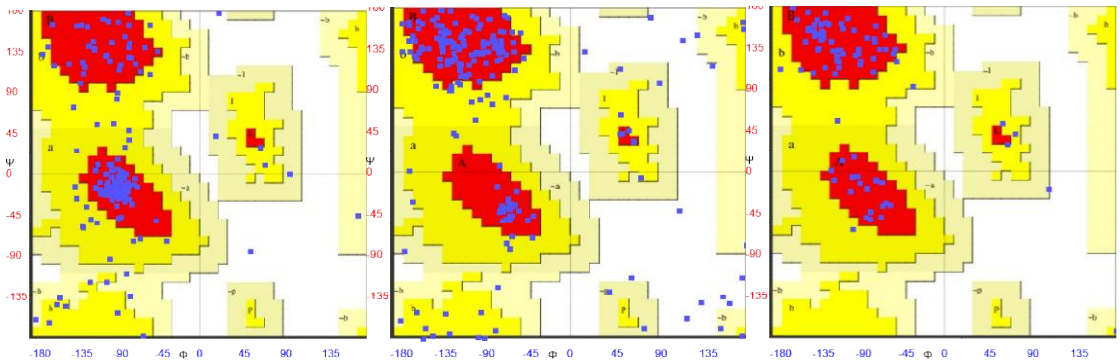
3.4 Homology Modelling of Coat-protein and Validation of 3D Model

Three different 3D models were obtained Model 1(Ball and Stick), Model 2(Spherical) and Model 3(Surface) from CBS server, Phyre2 Server and Swiss Model Server respectively. The model predicted using Swiss-Model (Fig. 2c) was considered as the best model based on structure validation scores (Table 3). The model structure obtained using Swiss-Model had 92.9% residues in the most favourable region of the Ramachandran plot (Fig. 3c) and showed Z-score for bond angles ,chi-1/chi-2 correlation and Ramachandran Z-score were 1.457,1.773 and -2.633 respectively which shows model quality was good.



a. Model 1 **b. Model 2** **c. Model 3**

Fig. 2. Homology model of CP of PLRV obtained by CBS (Model 1), Phyre2 (Model 2) and Swiss model server (Model 3)



a. Model 1 **b. Model 2** **c. Model 3**

Fig. 3. Ramchandran plot for coat protein structure obtained by CBS (Model 1), Phyre2 (Model 2) and Swiss model server (Model 3)

Table 3. Structure validation scores using SAVES v5.0 server for coat protein of PLRV

S. No.	Parameters	Model 1	Model 2	Model 3
1.	Number of residues in favoured region	143 (83.1%)	166 (82.6%)	78 (92.9%)
2.	Number of residues in allowed region	23 (13.4%)	18 (9.0%)	4 (4.8%)
3.	Number of residues in outlier region	6 (3.5%)	17 (8.5%)	2 (2.4%)
4.	Z-score for bond angles	1.688	1.457	1.457
5.	chi-1/chi-2 correlation, Z-score	-1.937	1.773	1.773
6.	Ramachandran Z-score	-5.779	-2.633	-2.633

Evaluation of model quality is one of the crucial step in homology modelling of protein. Once the 3D model is developed, the final model undergoes for the inspection using structure validation tools in order to confirm whether the model's stereochemistry is reasonably consistent with typical values found in crystal structures [18]. Persistent problems may suggest a problem with the alignment used to build the model; manual adjustments to the alignment may be necessary, particularly in the loop areas, followed by a rebuilding of the model. Structural analysis and verification server (SAVES) were used for evaluation of model quality. In the present work, we have taken an attempt in order to develop 3D- model of the coat protein of Potato Leaf Roll Virus isolated from Bihar region. The model was developed using three different homology model servers viz; CBS, Phyre2 and Swiss model server.

The model developed by Swiss-Model server (Model 3) was found the best as compare to the other models. It was having maximum core region (92.9%) and less disallowed region (2.4%) with minimum energy. The average Z score, chi-1/chi-2 correlation Z-score, Ramachandran Z-score represented in Fig. 3 also suggest the good quality of the predicted 3D model.

4. CONCLUSION

Pattern of the nucleotide diversity suggest the evidence regarding influence of geographic location on variability based on coat protein gene sequence. Homology modelling provide useful information about the structure of proteins of interest. Structure of unknown proteins can be identified on the basis of amino acid sequence pattern matching of both known and unknown proteins. By selecting homologous proteins showing maximum sequence similarity with the unknown protein for homology search a protein structure may be predicted that could be similar. Protein structures with more turn and coils might be expected to have an effect on rate of evolution. The preliminary information generated in this study may aid in establishment of broad-spectrum control strategies against PLRV. Further we are in the process of characterizing PLRV collected from different districts of Bihar in order to find out possible solution to control this disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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