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HOMOLOGY MODELLING OF PR1 PROTEIN IN RESPONSE TO *XANTHOMONAS ORYZAE PV ORYZAE* AND *RHIZOCTONIA SOLANI*: A STRATEGIC APPROACH FOR MITIGATING PATHOGENESIS THREATS IN *ORYZA SATIVA* SUBSP. INDICA.

Showmy K. S.[1*], Yusuf A.[2]

1. Department of Botany and Computational Biology, St. Aloysius College, Elthuruth, Thrissur, 680611, India.
2. Inter University Centre for Plant Biotechnology, University of Calicut - 673635, India.

ABSTRACT: Agriculture systems in Kerala are famous for large number of biotic and abiotic stress resistant native varieties of rice. Pesticides play a vital role in controlling the rice diseases. By effectively targeting the Pathogenesis Related proteins (PR proteins) helps to control the spread of diseases in rice fields. By predicting the three-dimensional structure of PR1 protein based on their homologous, we gain insights into their interactions and functions. For the study, nine local rice varieties from North Kerala were used for finding out the resistance of *Xanthomonas oryzae pv. oryzae (Xoo)* and *Rhizoctonia solani (R.solani),* most common biotic constraints of rice which causes yield loss due to bacterial blight and sheath blight respectively. We observed that *Chennellu and Kayma* are the most prominent resistant rice cultivars against *Xoo* and *R. solani* respectively. Total RNA of these two rice cultivars were isolated and one step RT-PCR was performed by designed PR1 primers. It was observed that C*hennellu* and K*ayma* rice cultivars expressed PR1 protein in their defense mechanism. The importance of PR1 in conferring resistance to fungal pathogen is well known, present study investigated that the PR1 gene was also expressed during resistance against bacteria. The partial PR1 cDNA isolated by RT-PCR was sequenced and submitted to NCBI (ID: KP826795.1 & KP257093.1) which was determined to encode a PR protein. Homology modelling of PR1 proteins were done by MODELER program from Discovery studio 4.0 with 1CFE as template and submitted to PMDB. Identified PR1 proteins serve as invaluable compounds for guiding researchers in the rational design of pesticides for mitigating the impact of pathogenic threats in agriculture.

Keywords: PR1 protein, Homology modelling, Pesticides, Rice diseases, Agriculture, defense mechanism

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Corresponding Author: Dr. Showmy K. S.* Ph.D.
Department of Botany and Computational Biology, St. Aloysius College, Elthuruth, Thrissur, 680611, India. Email Address: showmyreshin@gmail.com

1.INTRODUCTION

The development of innate resistance mechanism is a hereditary trait in plants. The defence mechanisms for protection of plants against pathogens develop cellular responses by induction of diverse proteins. Pathogenesis-related (PR) proteins are among them and have been playing a vital role in the disease resistance response. These PRs are defined as the proteins coded by the host plant which are induced specifically in pathological or related situations [1], [2]. Induction of PR proteins plays a major role in adaptation of biotic and abiotic stress conditions. PR proteins were first reported in tobacco after TMV infection by two independently working groups [3], [4]. They initially called it as “b” proteins. These proteins are commonly induced in resistant plants, bearing a hypersensitive necrotic response (HR) to pathogens of viral, fungal and bacterial origin. Later on it was shown that b-proteins are induced not only in resistant, but also in susceptible plant – pathogen interactions, as well as in plants subjected to abiotic stress factors. Then the name “pathogenesis-related” proteins were proposed by Antoniw *et al* from different tobacco cultivars [5]. PR proteins were shown to be induced by pathogens, wounding, fungal cell wall elicitors, ethylene, UV light, heavy metals, etc [6]. Five main groups of PRs (PR-1 to PR-5) were reported and characterized by both molecular and molecular-genetic techniques in tobacco, which are numbered in order of decreasing electrophoretic mobility [7]. Each group consists of several members with similar properties. Then eleven families (PR-1 to PR-11) were recognized and classified in tobacco and tomato [2]. Three novel families (PR-12, PR-13 and PR-14) were recognized in radish, Arabidopsis and barley, respectively [8]. PR1 protein was the first pathogenesis related group of protein in this family [9]. It is a dominant group that is induced by pathogens, and is commonly used as a marker for SAR [8] and also for activation of hypersensitive response (HR)-mediated defense pathways. 32 type PR-1 genes are found in rice genome [10] that are distributed among seven chromosomes and the majority are located on chromosome 7 [11]. Recent studies states that the expression of PR1 proteins showed its general stress response in some grapevine culture systems [12]. Several studies have done to elucidate the function of PR1 proteins but with not much success since the discovery in 1970. Even though its biological activity is still obscure, reports have shown its antifungal activity towards the host plants. PR1 was normally produced on pathogen attack as shown up to 2% of the total leaf protein in tobacco [13]. It belongs to small multigene families [8]. PR1a gene was

expressed in transgenic tobacco plants and shows high tolerance to *Phytophthora parasitica* var. *nicotianae* and *Peronospora tabacina* [13] and exhibited direct antifungal activity in tomato plants [14]. The acidic *N. tabacum* PR-1a was the first to be purified and characterized [5] and has been taken as the type member of the PR-1 proteins. The structure of tomato PR-1b (P14a) was solved by Nuclear Magnetic Resonance and found to represent a unique molecular architecture. This protein contains four α-helices (I-IV) and four β-strands (A±D) arranged antiparallel between helices I, III and IV and II, respectively. Quality and quantity of rice is reduced by wide range of micro organisms including bacteria, fungi and viruses. The most common biotic constraints of rice, bacteria and fungi, are *Xanthomonas oryzae pv. oryzae* (*Xoo*) and *Rhizoctonia solani* (*R. solani*) which causes yield loss due to bacterial blight and sheath blight respectively and plays crucial role in decreasing the productivity and quality of rice grains [15]. Usually, pesticides and fungicides are used to treat blight and blast diseases but they are not advised since they harm our environment. Hence, genomics and proteomics approaches show promise for particular disease control by identifying key insight genes and developing the host resistance plants or naturally developed bio-pesticides [16]. The effective chemical control measures are not yet available against these diseases. The uncontrolled use of agro chemicals to prevent the diseases is environmentally hazardous and cause depletion of soil fertility. The resistance of the variety will be broken by the virulent strains of the pathogen. Thus the management through the application of eco-friendly plant products seem useful. Hence there is need for biological products which act as agents against the attack of bacterial and fungal pathogens by reducing the chemical usage in soil or improve the host defense mechanism by learning the defense related genes. In order to prevent the use of chemicals, we are planning to introduce the importance of pathogenesis related protein in paddy cultivation. Homology modelling of PR1 proteins will be dreadfully supportive for the development of biological solution for bacterial blight and blast diseases in rice. Even though PR1 protein was discovered in 1970s, the function of this particular protein is still obscure. Since the structure is familiar henceforth the function of protein can be predicted.

2. MATERIALS AND METHODS

1. Plant Material:

Seeds of native rice cultivars were collected from Wayanad, North Kerala, India. Adukkan, Ayiramkana, Chennellu, Chomala, Gandhakasala, Kayma, valichoori, veliyan and Thondi were used for the study. Seeds of these rice varieties were disinfested with 0.1% $HgCl_2$ (mercuric chloride) for 1 min, followed by washing with sterilized distilled water and kept for germination in disposable petriplates. 45-day old plants were used for the study.

2. Inoculum preparation:

2.1 Bacterial pathogen

Xanthomonas oryzae pv. *oryzae,* causing bacterial leaf blight, was collected from Kerala Agricultural

University, Vellanikara and maintained on nutrient agar. Analytical grade chemicals from Himedia were used. Two days old cultures of *Xanthomonasoryzae*pv. *oryzae* (*Xoo*) having concentration of approximately 10^8 CFU/mL ($0.5OD_{600}$nm) were inoculated in to nutrient broth medium and were incubated on a rotary shaker (130 to 140 rpm) for 48–72 h at 28ºC. The supernatant of *Xoo* broth culture after centrifugation at 5000rpm for 10min were inoculated in different volume such as 2.5µl, 5µl, 10µl and 20µl. Inoculation was carried out on manually created wounds on the plants with sterile forceps and conducted mock treatment with distilled water.

2.2 Fungal strain

*Rhizoctonia solan*i causing sheath blight was procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh and subcultured and maintained on Potato Dextrose Agar (PDA) medium (Himedia). Liquid cultures were initiated by inoculating a piece of fresh mycelia in 200 ml of potato dextrose broth (PDB) medium in a 250-ml Erlenmeyer flask. The cultures were incubated on a shaker (130 to 140 rpm) for 7 to 10 days in dark; the cultured mycelia were harvested and cut into small mycelial balls (approximately 0.5 cm in diameter) with forceps.

3. Determination of Lesion length

Within 4-7 days after incubation, plantlets were planted in field having pond soil and cow dung in the Botanical Garden of Calicut University. The experiment was laid out in randomized block design in plots of 6 x 4 m^2 size. Separate plots were used for different isolates. 45-day old rice plants were used for bacterial challenge. The study on morphological variability has been carried out by analyzing the leaf lesion length of nine cultivars against the pathogen. The lesion length from the cut leaf tip was measured in centimetres (cm) after 21 days of inoculation. Hypersensitivity reactions were categorized according to lesion length using standard IRRI procedure for bacterial blight. The disease index (DI) of sheath blight was calculated as lesion length divided by plant height and multiplied by 9 [17]. The average of 10 seedlings was considered as an observation. According to DI against each isolate, the resistance to sheath blight was graded into 5 levels, namely resistance (R), moderate resistance (MR), moderate susceptibility (MS), susceptibility (S), and high susceptibility (HS) [18].

4. Preparation of RNA

Total RNA of selected resistant and susceptible rice cultivars of two different treatments (Bacterial & fungal) in 0^{th} day as control and 24h, 48h, 72h as treatments were extracted with buffer contained 100mM Tris-HCl (pH 8.0), 25mM Na-EDTA (pH 8.0), 2M NaCl, 2% CTAB and 2% PVP and followed by LiCl-Phenol extraction method. Thus RNA obtained was quantified by using a Nanodrop spectrophotometer and resolved in 1.5 % (w/v) formaldehyde-agarose gel.

5. **PCR amplification of cDNA encoding PR1 protein:**

Primers used in this study were designed by Primer-BLAST software of NCBI. The coding sequence of PR1 primer was amplified from rice total RNA by OrionX One step RT PCR kit (Origin

Diagnostics and Research, Karunagapalli, Kerala, India) using primers 5'-GCATCGAAAATGGCAACCTCC-3', 5'-CGGCTGACGGCTTTATTCCC-3'. PCR was carried out in a Mastercycler Pro S (Eppendorf). The reaction initially performed was the reverse transcription and was carried out at 50ºC for 30min. Then the reaction cycles were programmed denaturation at 95ºC for 15 min and 40 cycles of 95ºC for 20s; temperature gradient (61.1, 62.4, 63.7, 64.9 and 65.9ºC) for 40s as annealing and extension at 72^0C for 45 s; and a final extension at 72ºC for 5min. Optimum amplifications was obtained at 63.7ºC, further amplifications were carried out at 63.7ºC annealing temperature. PCR products were fractionated on a 1% agarose gel and the expected size bands were cut out and purified using the MiniGel DNA Purification Kit (Origin Diagnostics and Research, Karunagapalli, Kerala, India).

6. DNA Sequencing of PR1 cDNA:

The PCR products of *Xanthomonas oryzae pv. oryzae* and *Rhizoctonia solani* treated resistant rice cultivars were sequenced as a charge basis from Scigenom Labs Private Ltd, Cochin. PR1 nucleotide sequence against *Xoo* and *R. solani* were submitted to the NCBI database. Nucleotide sequences of *Xanthomonas oryzae pv. oryzae* and *Rhizoctonia solani* treated resistant rice cultivars were deposited in NCBI as KP826795 & KP257093 respectively.

7. Protein sequence analysis

The nucleic acid sequences obtained were translated into its corresponding amino acids. The primary structure of PR1 proteins was studied by ProtParam tool of Expasy Proteomic Server. Different physicochemical properties such as molecular weight, theoretical isoelectric point, aliphatic index and grand average hydropathy (GRAVY) were calculated.

8. Secondary structure prediction & Domain analysis

Protein secondary structure predictions were performed at the NPS@ server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_server.html) using the consensus prediction with a combination of the methods of DMP, DSC, GOR4, HNNC, PHD, Predator, SIMPA96 and SOPM [19]. CATH is used for deriving a novel hierarchical classification of protein domain structures. The four main levels of classification are protein class (C), architecture (A), topology (T) and homologous superfamily (H) [20]. The Interpro Scan tool was used to understand the protein family, super family, and domain arrangement within the protein. Conserved domains of the PR1 protein were explored by using Pfam. The signal peptide was predicted by SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/) [21].

9. Molecular modelling

The PR1 protein sequences of *Xoo* and *R. solani* treated resistant rice cultivars were used as query sequences in the homology modelling process. Template from known protein structures based on sequence similarity was predicted using BLASTP program against PDB database. Tree-based consistency objective function for alignment evaluation (T-COFFEE) is a multiple sequence

alignment (MSA) program and was used to detect the conserved regions. MODELLER in co-operated in Discovery Studio Visualizer 4.0.0.13259 was used to build protein models according to the comparative protein modelling methodology. The model was refined by CHARMm force field in DS, which provides powerful mechanics and dynamics protocols for studying the energetics and motion of molecules. The pairwise 3-D structure superimposition of the elucidated model of PR1 proteins with its closest structural homologue was carried out in this same platform.

10. Model Validation

A number of tools were used to evaluate the internal consistency and reliability of comparative modelled structure of PR1. PROCHECK was used to assess the stereo chemical quality of the model which quantifies the residues in available zones of Ramachandran plot [22]. VERIFY-3D program was used to determine the compatibility of the atomic model (3D) with its own amino acid sequence (1D) [23]. ERRAT tool was used to check the overall qualities of the modelled structure [24]. ERRAT were carried out using Structural Analysis and Verification Server (SAVES) (http://nihserver.mbi.ucla.edu/SAVES/). ProSA tool, to check the native protein folding energy of the model by comparing the energy of the model with the potential mean force derived from a large set of known protein structures, was employed in the refinement and validation of the modelled structure [25]. Next the constructed structure was yet again assessed its reliability using QMEAN score by QMEAN server for model quality estimation of SWISS MODEL server [26]. DaliLite computes the root mean square deviation (RMSD) between the Cα-atoms and all other atoms of the homology model and template. The homology model of the 3-D structures of PR1 of *O. Sativa* treated with *Xoo* and *R. Solani* were submitted to PMDB (http://mi.caspur.it/PMDB/) [27] which were assigned under PM0080067 and PM0080068 respectively. They made available in PMDB for public reference.

3. RESULTS AND DISCUSSION

Collected 9 rice cultivars were tested to find out the resistance and susceptibility against bacterial blight and sheath blight. Bacterial blight (BB) caused by *Xanthomonas oryzae pv. Oryzae* is a major seed-borne pathogen of rice and BB of rice causes considerable losses in all cultivars of rice in India. Effectors of Gram-negative plant pathogens are delivered into plant cells via a type III secretion system (T3SS) and Type II secretion system (T2SS). The T3SS consists of a Hrp pilus, resembling the flagellar biosynthetic complex, but producing a needle-like appendage hence the T3SS is encoded by hrp genes. Apart from hrp gene-encoded T3SS, T2SS (Type II secretion system) are found to the virulence of phytopathogenic bacteria [28]. T2SS consists of several degradative enzymes such as pectatelyases, cellulases, xylanases and proteases and play important roles in the interaction of *Xoo* with its host [29], [30]. Thus the rice leaves were inoculated with supernatant of *Xoo* and inoculated leaves of rice plants showed the yellow lesions were used as the symptom of bacterial blight. The length of the lesion was recorded after 21 dpi. From the 4 different volumes

(2.5µl, 5µl, 10 µl and 20µl), chennelu showed high resistance to *Xoo* and Ghandakasala shows susceptibility in both 10 µl and 20µl volume (Figure 1).

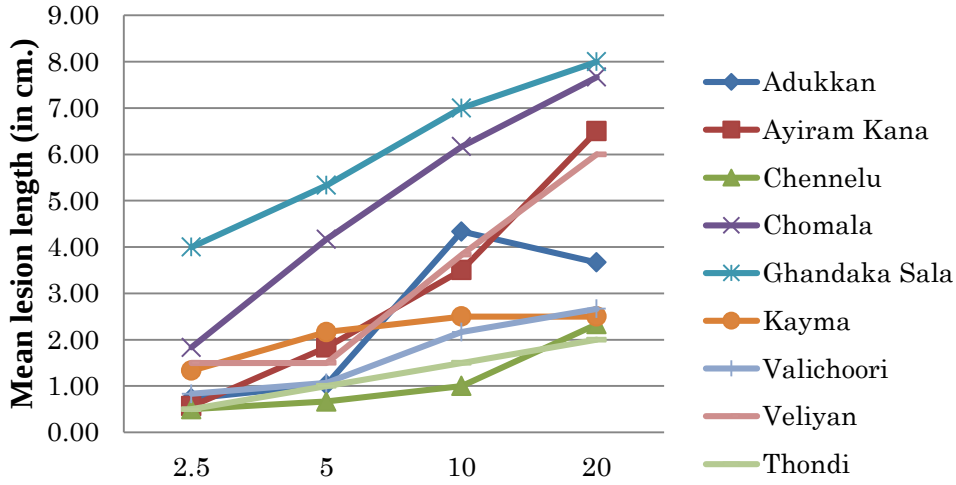


Figure 1: Relationship of *Xanthomonas oryzae pv. oryzae* inoculum concentration with bacterial leaf blight severity.

10 µl of supernatant solution of *Xoo* are selected for production of PR proteins. On the other hand, in the case of *R. solani*, the reaction was varied. Here also resistance is not affected by plant height, heading date, and plant compactness [31]. Hence the disease index has to calculate based on the lesion length only. Kayma cultivars showed resistance and *Chennellu* shows susceptibility to *R. solani* (figure 2).

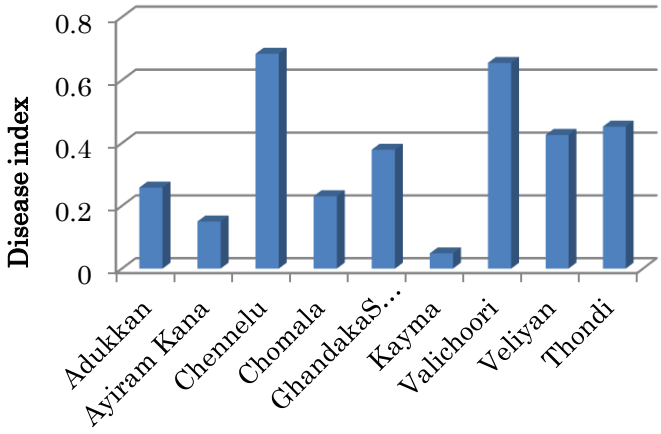


Figure 2: Relationship of *Rhizoctonia solani* with sheath blight severity.

Isolation of cDNA for PR1 protein

Total RNA of both resistant and susceptible rice cultivars of pathogens treated in 0^{th} day as control and 24h, 48h, 72h were extracted by standard CTAB method with some modification. Amplified PCR product was obtained from one step RT PCR (figure 3). Ten µg of total RNA were applied to

each lane in a 1% agarose gel.

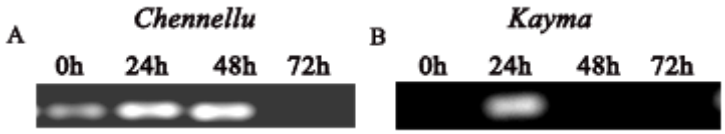


Figure 3: RT-PCR validation of PR1 gene after pathogen stress treatments. Intensity of mRNA level of constitutive resistance and constitutive susceptible at 0, 24, 48, 72h post day inoculated plants. 0h served as control. Total RNA was isolated from *Xoo* treated leaves (A) and *R.solani* treated leaves (B) of 45d old seedlings after treatment for the indicated times; Ten µg of total RNA were applied to each lane in a 1% agarose gel.

Upon sequencing, the amplified fragments were found to contain a reading frame beginning with methionine, followed by the amino-terminal residues determined by protein sequencing. The OsPR1 cDNA is 864 bp long which contains a putative 450 bp open reading frame encoding 149 amino acid residues and 717 nucleotides which also contains 450 bp open reading frame encoding 149 amino acid residues in KP826795 & KP257093 respectively.

Pairwise alignment of these two-protein sequence by blastp will shows 99% identity and Proline(P) in KP826795 is replaced by Alanine(A) in KP257093 on 75th position of protein sequences.

Protein sequence analysis of PR1

PR1 proteins were studied for physicochemical properties. The ProtParam tool computed a molecular weight of 15.69KDa for KP826795 and 15.67KDa for KP257093. The isoelectric point (pI) value of proteins was shown to 4.23. The aliphatic index (AI) is defined as the relative volume of a protein occupied by aliphatic side chains such as alanine, valine, isoleucine, and leucine. Aliphatic index of these two PR1 were 55.77 and 56.44. The Instability index was 47.05 and 45.76 and notified the protein as unstable, Grand average of hydropathicity (GRAVY) was -0.366 and -0.343 (indicates high affinity with water, as value ranges are low) for KP826795 & KP257093 respectively.

Secondary structure prediction

Secondary structure consensus prediction revealed that PR1 possess 26.85% & 28.86% Alpha helix, 52.35% & 48.99% random coil, 20.13% & 20.81% extended strand and 0.67% & 1.34% ambiguous states was found in KP826795 and KP257093 respectively. Analysis of the structural families generated by CATH reveals that the PR1 structure confirmed with superfamily Pathogenesis related protein 14a (3.40.33.10) and functional family as Golgi associated Pathogenesis Related 1 (GAPR-1). InterProscan showed that PR1 proteins may undergo Cysteine-rich secretory protein family known to be CAP **(c**ysteine-rich secretory proteins, **a**ntigen 5, and **p**athogenesis-related 1 proteins) family. 6 cys residues are found in this particular PR1 protein. Pfam shows the alignment region of CAP domain ranges from 13 to 137. SignalP4.1 predicted PR1 without any signal peptide cleavage sites.

Homology modelling of PR1

A search with BLASTP against PDB was assisted to retrieve homologous template structures for the 149 amino acid residues long target PR1 proteins. BLAST search revealed three putative templates (PDB id: 1CFE, 2DDA and 2DDB) of identity with the target sequence, as shown in Table 1.

Table 1: Template selected for homology Model Building of PR1 from BLAST Search against PDB

PDB ID	Name of Protein	Source	Chain	Percentage of Identity	Query Range	E-Value	Score
1CFE		Solanum lycopersicum	A	48%	95%	1e-36	126
2DDA	Pseudechetoxin	Pseudechis australis	A	30%	98%	1e-12	62.8
2DDB	Pseudecin	Pseudechis porphyriacus	A	30%	96%	4e-12	61.2

NMR structure Chain A of *Solanum lycopersicum* P14a (PDB: 1CFE) having 135 amino acid residues shows the best identity of 48% with the target sequences. Multiple sequence alignment between the target and template protein sequences using structural information by Tcoffee tool revealed that the amino acids represented with the asterisk symbol are highly conserved and has good identity [32]. The sequence alignment between the target and template showed 96 consensus regions (Figure 4).

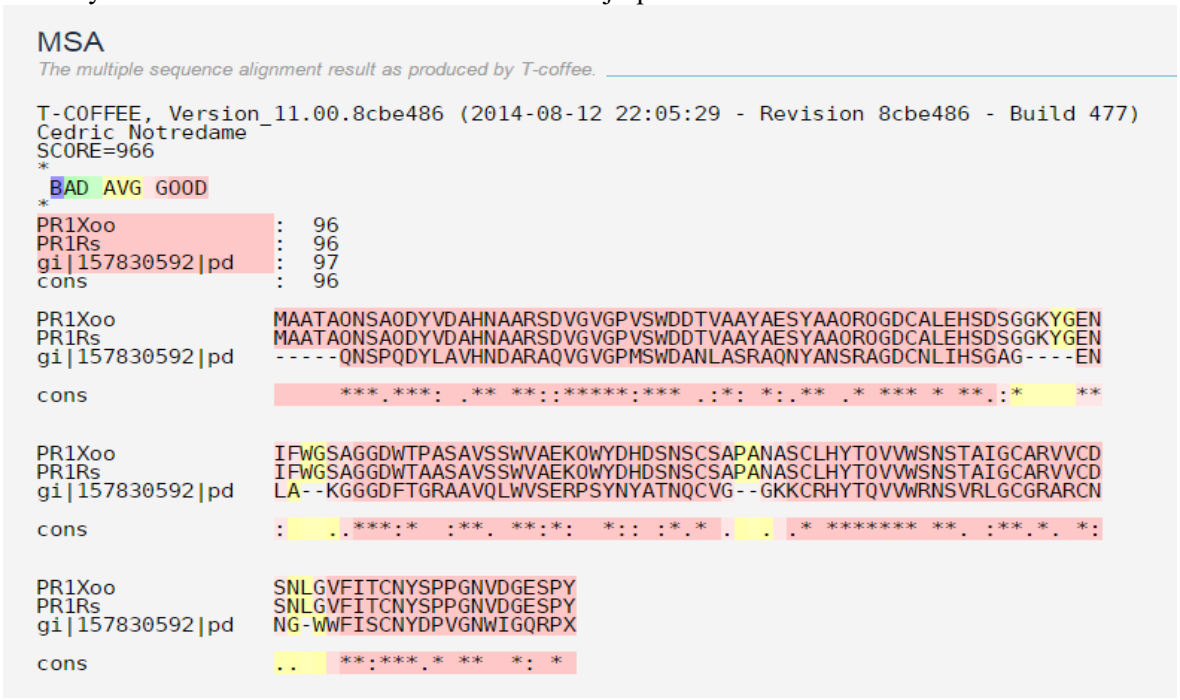
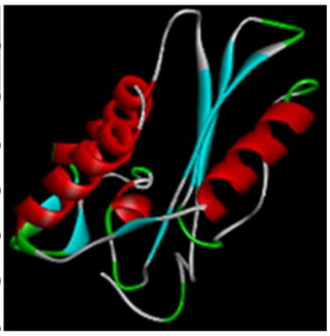


Figure 4: Multiple sequence alignment of the targets and template predicted by T-COFEE. The symbol "*" indicate highly conserved (identical) residues, ":" indicate high similarity and "." Indicate low similarity.

Five different 3D models of PR1 were generated by Modeller in cooperated in Discovery Studio 4.0.0.13259 based on the target-template alignment which employs extraction of spatial restraints from two sources (i.e., homology derived and CHARMm force field derived) [33], [34]. Model005 has lowest PDF energy (5341.2905) and DOPE score as -12651.099609 and considered as best model of KP826795. Moreover Model 3 having lowest energy, 1173.2363 and DOPE score -12170.85 were considered as the best model of KP257093 (figure 5).

Model Scores			
Name	PDF Total Energy	PDF Physical Energy	DOPE Score
targetPR1Xoo.M0005	5341.2905	391.41898	-12651.099609
targetPR1Xoo.M0001	5342.9697	387.3479219	-12782.760742
targetPR1Xoo.M0003	5415.4854	408.1029595	-12661.617188
targetPR1Xoo.M0004	5426.1035	422.0313481	-12681.297852
targetPR1Xoo.M0002	5449.0508	409.7509099	-12852.065430

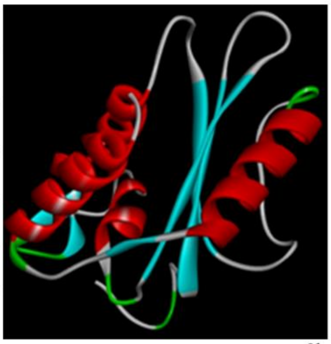
1a



1b

Model Scores			
Name	PDF Total Energy	PDF Physical Energy	DOPE Score
Target2.M0003	1173.2363	529.842202154	-12170.849609
Target2.M0002	1279.9896	590.458292	-12268.726563
Target2.M0005	1308.4966	571.597596300001	-11938.501953
Target2.M0004	1454.5110	662.1490627	-11843.297852
Target2.M0001	1530.0293	689.858703	-11872.562500

2a



2b

Figure 5: Summary and 3D modelled structure of KP826795 (1a and 1b); Summary and 3D modelled structure of KP257093 (2a and 2b).

The pairwise 3-D structure superimposition of the elucidated model of PR1 protein with its closest structural homologue was shown in figure 6.

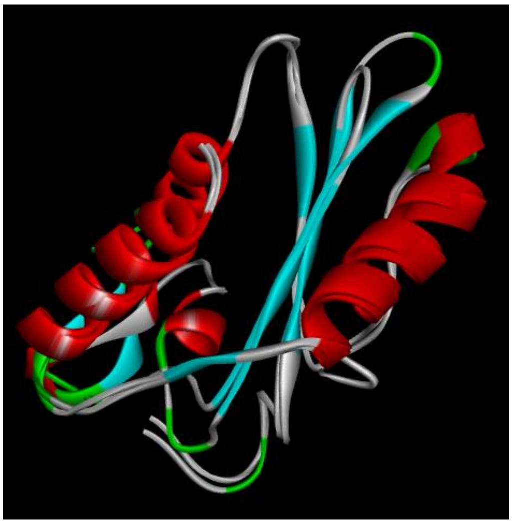


Figure 6: Superimposed image: Three-dimensional structural superimposition of PR1 model with template 1CFE

Validation of modelled structure

To check the best models, we have to go for validation processes. PROCHECK quantifies the residues fall in the available zones of Ramachandran plot, which was used to check the reliability of the backbone of torsion angles of the model (Figure 7).

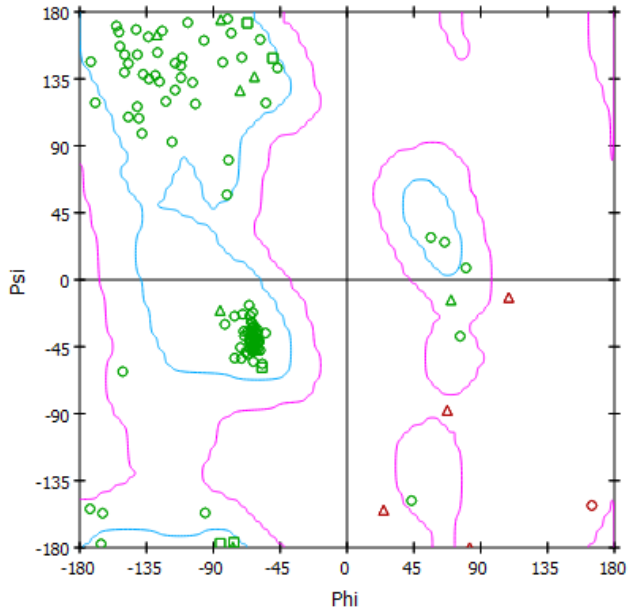


Figure 7: The stereo chemical validation of the hypothetical model by Ramachandran plot generated by ProCheck.

Ramachandran plot analysis for the modeled protein of PR1 (KP826795) showed that 87.8% residues fell in the most favored regions, 10.6% residues were in additional allowed regions, and 0.8% residues were in the generously allowed and disallowed regions. In the same way, modeled protein of PR1 (KP257093) showed that 85.5% residues fell in the most favored regions, 12.1% residues were in additional allowed regions, 0.8% residues were in the generously allowed and 1.6% in the disallowed regions.

The VERIFY-3D results of the PR1 models showed 100% of the amino acids had an average 3D-1D score of >0.2, and verified score of KP826795 and KP257093 was 66, which was greater than the expected score that ranges from 29.314 to 65.1422 (Table 2), indicating the reliability of the proposed model.

Table 2: Verified score of PR1 proteins

Name	Verify Score	Verify Expected High Score	Verify Expected Low Score
Target2	66	65.1422	29.314

ERRAT is a so-called “overall quality factor” for non-bonded atomic interactions, and higher scores mean higher quality protein [24]. The normally accepted range is >50 for a high-quality model. ERRAT score of our models are 61.765 (KP826795) and 55.882 for KP257093. Z-score RMS value of ~1.0 indicates good resolution of structures.

The energy profile of the model and the Z-score value were obtained using ProSA server that calculates the interaction energy per residue using a distance-based pair potential. The ProSA analysis of the models of PR1 achieved a Z score of -6.44 (KP826795), -6.25 (KP257093) and that of template was -5.67 (figure 8).

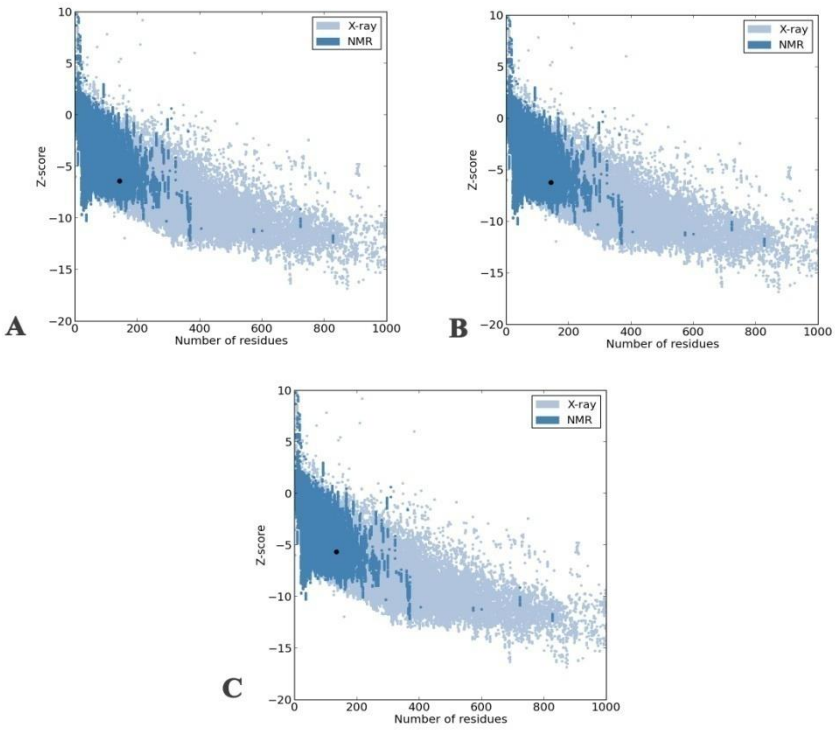


Figure 8: The refinement of the hypothetical model by ProSA server. The black dot represents Z-Score of the model in comparison with NMR structure of the template A) ProSA analysis of KP826795 and represents Z score of -6.44 B) ProSA analysis of KP257093 and represents Z score of -6.25 C) ProSA analysis of template and represents Z score of -5.67.

QMEAN score calculates the 'degree of nativeness' of the structural features observed in a model and by describing that a given model is of comparable quality to experimental structures [35]. QMEAN score of KP826795, KP257093 and template were 0.733, 0.705 and 0.686 respectively.

DISCUSSION

1. Identified the degree of pathogenecity against bacterial blight and sheath blight

The present study discovered that *Chennellu* and *Kayma*, two important native rice cultivars in the region of Wayanad, North Kerala, are the most resistant varieties against bacterial blight and sheath blight respectively. No information was available for evaluating wild relatives against *Xoo* and *R. solani* from Kerala. Pathogenecity test was carried on 21 dpi of seedlings of all randomly selected leaves of plants. Age of plant greatly influenced the varieties that were commonly susceptible at seedling stage and these same varieties showed more prominent resistant response in the later stages. Here we selected seedling stage for their study of resistance against the particular diseases. The testing in seedlings would be sufficient for preliminary screening of a large number of rice cultivars against BB disease [36]. Disease reactions were categorized according to lesion length. The lesion developed on *Chennellu* and *Kayma* were recorded with mean leaf lesion length 0.5cm exhibiting its resistant reaction against bacterial blight and sheath blight respectively. Standard Evaluation System IRRI declared that data were recorded to identify the degree of pathogenecity on 0-4 rating

scale [17]. Other reports also classified that the lesion length 0 to 6 cm as resistance (R) and more than 6 cm as susceptible (S) [37].

2. RT-PCR validation of PR1 gene after pathogen stress treatments

Intensity of mRNA level of PR1 gene was increased in both *Chennellu* and *Kayma* resistant cultivars during the 48h and 24h post inoculation on leaves respectively. On the other hand expression of PR1 gene was also found by wounding [6]. During studies of transcriptional profiling, RT-PCR validation of the expression of calcium-binding EF-hand (AK058544) and protein phosphatase 2C-like (AK100561) genes indicated an increase in their expression levels after bacterial infection in IET8585 [38]. Anti-fungal activity of PR-1 was reported in transgenic tobacco and tomato [13], [14]. PR1 protein normally known to be an anti-fungal protein, but here we declared that PR1 gene was also expressed in seedlings of *Xoo* treated native rice cultivars. PR1 showed enhanced expression in *Xa21*-mediated resistance responses at later stages after inoculation with *Xoo [39]. Since they have a role in anti-bacterial pathogenecity, we suggest that PR1 may be called as anti-microbial PR protein.*

3. PR1 structure analysis

The proteins modelled from rice plants treated with *Xoo* and *R. solani* had a very good sequence identity only with a single base pair change. Proline(P) in KP826795 is replaced by Alanine(A) in KP257093 on 75th position of protein sequences. In this study, nucleotide sequence of PR1 gene shows 717bp and 864bp. Somssich et al estimated in their report that the lengths of the PR1 mRNAs to be 750-900 nucleotides [38]. The ProtParam tool calculated the molecular weight of PR1 ranges from 15.67KDa (KP257093) to 15.69KDa (KP826795). Molecular mass of PR1 is 15–17 kDa [9]. Isoelectric point value was showed to 4.23, indicating its acidic nature (pI <7 show acidic nature whereas pI >7 indicate basic nature). PR1 cDNA (*OsPR1a*) was isolated from jasmonic acid (JA)-treated rice seedling leaf and reported as acidic protein having the P^I value of 4.4 [41]. NtPR1a, b, and c genes for acidic proteins were reported in tobacco (*Nicotiana tabacum*). Basic nature of PR1 also were short C-terminal extensions have mostly been characterized as basic proteins, and these sequence additions reflect their slightly higher molecular weights [8]. Aliphatic index is considered as a positive factor for the increase of thermal stability of globular proteins [42]. Aliphatic index of these two PR1 were 55.77 and 56.44 which may be less stable for a wide range of temperature (relatively higher value shows greater stability). A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable [43]. The Instability index ranges from 45.76 to 47.05 classifies the protein as unstable. GRAVY indicates its high affinity with water, as value ranges are low for KP826795 & KP257093 respectively. InterProscan revealed that these proteins are rich in cysteine residues. 6 cys residues in PR1 may forms 3 disulphide linkages. The mature proteins are mostly contain six conserved cysteine residues forming disulphide bridges, and suggesting that all proteins share the same a-b-a sandwich structure

with tomato PR-1a [8], [44].

4. Comparative modelling of 3D structure of PR1 proteins

The 3D structural models of the two PR1 proteins have been modeled using the homology modeling technique. Homology modelling for PR1 is based on the alignment with the NMR structure of 1CFE (*Solanum lycopersicum*) since the sequence identities between the templates and target structures were 48% which is sufficient for homology modelling [45]. The sequences were aligned with T-coffee shows consensus region between the targets and template and declared its similarity. For each protein, five models have been calculated by MODELLER [34] in cooperated in Discovery studio 4.0. All five models were ranked with their PDF energy and DOPE score. The model with the lowest PDF energy and DOPE score was considered as the best model. Model005 has lowest PDF energy (5341.2905) and DOPE score as -12651.09 and considered as best model of KP826795. Moreover Model 3 having lowest energy, 1173.23 and DOPE score -12170.85 were considered as the best model of KP257093. Model assessments using the program PROCHECK revealed that residues of PR1 KP826795, KP257093 were located in the most favoured regions of the Ramachandran plot are 87.8% and 85.5%, and in the additionally allowed regions are 10.6% and 12.1%, and the Ala102 and Asp32, Ser54, are located in the disallowed region respectively. The VERIFY-3D results of the PR1 models showed 100% of the amino acids had an average 3D-1D score of >0.2, indicating the reliability of the proposed model. The quality of our models of PR1 were further supported by a high ERRAT score of 61.765 (KP826795) and 55.882 for (KP257093), which indicates acceptable protein environment [24]. The quality of the structure was inspected with PROSA that allowed all residues in negative energy regions very similar to the template protein, and indicates a good quality model since the negative energy reflects reliability of the model. The QMEAN score also proved that the model predicted by SWISS MODEL was trustworthy because the score falls between 0 and 1.

4. CONCLUSION

Sequence analysis and homology modelling of “PR” of native rice cultivars in Kerala have not yet been attempted. Almost all native cultivars showed resistance to pathogens than the hybrid varieties. Here we recognized that *Chennellu* and *Kayma* are the most resistant rice cultivars against bacterial blight and sheath blight respectively. PR1 proteins are the initially identified PR proteins but shows uncertainty in their complete mechanism. Induction of PR1 gene in pathogen attack and also wounding indicates that this also help to disease resistance as well as the plant to adapt to the environmental stress. PR1 proteins analyzed from both cultivars treated with bacterial and fungal pathogens shows 99% identity. In this study, 3D structures of PR1 were developed using 1CFE as template which exposed most identity with the targets. Molecular model of PR1 proteins were predicted using homology modelling method which is reliable and it can be exploited for further function predictions. The present work is the first attempt in molecular characterisation of native

rice cultivars in Kerala against bacterial blight and sheath blight, and homology modelling of PR1 proteins. These all add relevance in the host-pathogen defense system.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

There is no conflict of interest exists.

REFERENCES

1. Antoniw JF, Pierpoint WS. Purification of a tobacco leaf protein associated with resistance to virus infection. Biochem. Soc. Trans. 1978; 6: 248-250.
2. Van Loon LC, Pierpoint WS, Boller TH, Conejero V. Recommendations for naming plant pathogenesis-related proteins. Plant Mol. Biol. Rep. 1994; 12(3), 245-264
3. Van Loon LC, Van Kammen A. Polyacrylamide disc electrophoresis of the soluble leaf proteins from Nicotianatabacum var. 'Sansum' and 'Sansum NN′. II. Changes in protein constitution after infection with tobacco mosaic virus. Vir. 1970; 40: 199-211.
4. Gianinazzi S, Martin JC. Hypersensibilité aux virus, temperature et protéines solubles chez le Nicotiana Xanthi n.c. Apparition de nouvelles macromolécules lors de la répression de la synthèse virale. C. R. Acad. Sci. Paris, 1970; 270D, 2383-2386.
5. Antoniw, JF, Ritter CE, Pierpoint WS, VanLoon LC. Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. J. Gen. Virol. 1980; 47(1), 79-87.
6. Grover A, Gowthaman R. Strategies for development of fungus-resistant transgenic plants. Curr Sci. 2003; 84(3), 330-340.
7. Edreva A. Pathogenesis-related proteins: research progress in the last 15 years. Gen Appl Plant Physiol. 2005; 31(1-2), 105-124.
8. Van Loon LC, Van Strien EA. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol. Mol. Plant Pathol. 1999; 55(2), 85-97.

9. Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S., Kauffmann, S., Geoffroy, P., Fritig, B. Plant 'pathogenesis-related'proteins and their role in defense against pathogens. Biochimie. 1993; 75(8), 687-706.
10. Van Loon LC, Rep M, Pieterse CMJ. Significance of inducible defense-related proteins in infected plants. Annu. Rev. Phytopathol. 2006; 44, 135-162.
11. Liu Q, Xue Q. Computational identification of novel PR-1-type genes in Oryza sativa. J. Genet. 2006; 85(3), 193.
12. Wielgoss A, Kortekamp A. Comparison of PR1 expression in grapevine cultures after inoculation with a host-and a non-host pathogen. Vitis - J. Grapevine Res. 2015; 45(1), 9.
13. Alexander D, Goodman RM, Gut-Rella M, Glascock C, Weymann K, Friedrich L, Ward E. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. PNAS 1993; 90(15), 7327-7331.
14. Niderman T, Genetet I, Bruyere T, Gees R, Stintzi A, Legrand M, Fritig B, Mosinger E. Pathogenesisrelated PR-1 proteins are antifungal; isolation and characterization of three 14-kilodalton proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against Phytophthora infestans. Plant Physiol. 1995; 108: 17±27.
15. Singh P, Verma RL, Singh RS, Singh RP, Singh HB, Arsode P, Singh PK. Biotic stress management in rice (Oryza sativa L.) through conventional and molecular approaches. New front. In stress Manag. For dur. Agri. 2020; 609-644.
16. Rao TB, Kalagatur, NK, Gali G, Boggula, VK, Upadhyayula, MK, Mangamuri UK. Genomics and transgenic approaches for sheath blight resistance development in rice. NIScPR. 2024; 60(12), 886-895
17. IRRI Standard evaluation system for rice. 3rd edition, IRRI, Philippines. 1988.
18. Ling W, Huang WW, Lian-Meng LI.U., Qiang, F. U., & Huang, S. W. Evaluation of Resistance to Sheath Blight (Rhizoctonia solani) in Partial Indica Hybrid Rice Combinations from Southern China. AAS. 2011; 37(2), 263-270
19. Combet C, Blanchet C, Geourjon C, Deleage G. NPS@: network protein sequence analysis. TIBS. 2000; 25(3), 147-150.
20. Orengo CA, Michie AD, Jones S, Jones DT, Swindells MB, Thornton JM. CATH–a hierarchic classification of protein domain structures. Struct, 1997; 5(8), 1093-1109.
21. Petersen TN, Brunak S, von Heijne G, and Nielsen H. SignalP 4.0: Discriminating signal peptides from transmembraneregions. Nat. Methods. 2011; 8, 785–786.
22. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Crystallogr. 1993; 26(2), 283-291.
23. Eisenberg D, Lüthy R, Bowie JU. VERIFY3D: Assessment of protein models with three-dimensional profiles. Methods Enzymol. 1997; 277, 396-404.

24. Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Prot. Sci. 1993; 2(9), 1511-1519.
25. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. Spec. Publ. 2007; 35(2), W407-W410.
26. Benkert P, Tosatto SCE, Schomburg D. "QMEAN: A comprehensive scoring function for model quality assessment." Proteins: Struct., Funct., Bioinf. 2008; 71(1):261-277.
27. Castrignanò T, De Meo PDO, Cozzetto D, Talamo IG, Tramontano A. The PMDB protein model database. Nucleic Acids Res. Suppl. 2006; 34(1), D306-D309.
28. Alfano JR, Collmer A. Bacterial pathogens in plants: life up against the wall. Plant Cell. 1996; 8, 1683-1698.
29. Ray SK, Rajeshwari R, Sonti RV. Mutants of Xanthomonas oryzae pv. oryzae deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. Mol. Plant-Microbe Interact. 2000; 13, 394-401.
30. Sun QH, Hu J, Huang GX, Ge C, Fang RX, He CZ. Type-II secretion pathway structural gene xpsE, xylanase- and cellulose secretion and virulence in Xanthomonas oryzae pv. oryzae. Plant Pathol. 2005; 54, 15-21.
31. Ling W, HUANG WW, Lian-Meng LIU, Qiang FU, HUANG SW. Evaluation of resistance to sheath blight (Rhizoctonia solani) in partial Indica hybrid rice combinations from Southern China. AAS. 2011; 37(2), 263-270.
32. Notredame C, Higgins DG, Heringa J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. J. Mol. Biol. 2000; 302(1), 205-217.
33. Sali A, Potterton L, Yuan F, Vlijmen H, Karplus M. Evaluation of comparative protein modeling by MODELLER. Proteins: Struct., Funct., Bioinf. 1995; 23(3), 318-326.
34. Brooks BR, Bruccoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. J. Comput. Chem. 1983; 4(2), 187-217.
35. Benkert P, Biasini M, Schwede T. Toward the estimation of the absolute quality of individual protein structure models. Bioinform. 2011; 27(3), 343-350.
36. Ou SH, Nuque FL, Silva JP. Varietal resistance to bacterial blight of rice. Plant Dis. Reptr. 1971; 55, 17-21.
37. Vega-Sanchez ME, Erselius LJ, Rodriguez AM, Bastidas O, Hohl HR, Ojiambo PS, Forbes GA. Host adaptation to potato and tomato within the US− 1 clonal lineage of Phytophthora infestans in Uganda and Kenya. Plant Pathol. 2000; 49(5), 531-539.
38. Kottapalli KR, Rakwal R, Satoh K, Shibato J, Kottapalli P, Iwahashi H, Kikuchi S. Transcriptional profiling of indica rice cultivar IET8585 (Ajaya) infected with bacterial leaf

blight pathogen Xanthomonas oryzae pv oryzae. Plant Physiol. Biochem. 2007; 45(10), 834-850.

39. Hou M, Xu W, Bai H, Liu Y, Li L, Liu L, Liu G. Characteristic expression of rice pathogenesis-related proteins in rice leaves during interactions with Xanthomonas oryzae pv. oryzae. Plant Cell Rep. 2012; 31(5), 895-904.
40. Somssich IE, Schmelzer E, Bollmann J, Hahlbrock K. Rapid activation by fungal elicitor of genes encoding “pathogenesis-related” proteins in cultured parsley cells. PNAS. 1986; 83(8), 2427-2430.
41. Agrawal GK, Jwa NS, Rakwal R. A novel rice (Oryza sativa L.) acidic PR1 gene highly responsive to cut, phytohormones, and protein phosphatase inhibitors. BBRC, 2000; 274(1), 157-165.
42. Atsushi IKAI. Thermostability and aliphatic index of globular proteins. J. Biochem. 1980; 88(6), 1895-1898.
43. Praveen M. Characterizing the West Nile Virus's polyprotein from nucleotide sequence to protein structure–Computational tools. JTUMED. 2024; 19(2), 338-350.
44. Selitrennikoff CP. Antifungal proteins. AEM. 2001; 67(7), 2883-2894
45. Fernandez C, Szyperski T, Bruyere T, Ramage P, Mösinger E, Wüthrich K. NMR solution structure of the pathogenesis-related protein P14a. J. Mol. Biol. 1997; 266(3), 576-593.