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RESEARCH

In silico analysis of cloned brown planthopper genes unveiled OsJ_28113 as a key regulator in triggering resistance response in rice

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Abstract

Brown planthopper (BPH) is a highly destructive insect pest of rice, causing significant yield loss. Due to its constantly evolving nature, continuous analysis of BPH's protein domain-interacting partners is essential. In the present study, in silico approach was followed to predict 3-D structure of cloned BPH resistant proteins (*Bph6*, *Bph9*, *Bph14*, *Bph17*, *Bph18*, *Bph26*, *Bph29* and *Bph32*) using a comparative modelling approach and their interaction studies. The interactome analysis revealed a key regulator, OsJ_28113, responsible for transducing extracellular signals into intracellular responses, potentially aiding in activating proteins that provide resistance against BPH. The proposed model provides insights into the structure and active sites of these proteins, offering opportunities to develop novel strategies for BPH control in rice plants. The molecular profile analysis revealed that BPH resistance genes containing the CC-NBS-LRR domain have varying lengths of amino acid chains ranging from 1082 for *Bph30* to the longest (2024) for *Bph6*. *Bph26* and *Bph18* demonstrated high sequence similarity containing NB-ARC and LRR domains. The secondary structure prediction results anticipated that all the proteins, except *Bph30*, are cytoplasmic and soluble. The in silico findings support the notion that variability in resistance genes is a result of ongoing evolutionary interactions between plants and insect pests. Additionally, the study uncovered higher ligand binding affinities towards jasmonic acid compared to salicylic acid, paving the way for further research on receptor-ligand recognition and signalling mechanisms against rice planthoppers.

Keywords Brown planthopper · In silico · 3-D · CC-NBS-LRR · Jasmonic acid · Salicylic acid

Abbreviations

3-D	Three-dimensional
QTL	Quantitative trait loci
CC-NBS-LRR	Coiled-coil, nucleotide-binding site and leucine-rich repeat
SCR	Short consensus repeat
NCBI	National Center for Biotechnology Information
RGAP	Rice Genome Annotation Project
BLAST	Basic Local Alignment Search Tool
BAC	Bacterial artificial chromosome
FASTA	Fast-all
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
NMR	Nuclear magnetic resonance spectroscopy
CRISPR	Clustered regularly interspaced short palindromic repeats
RNAi	RNA interference

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Introduction

Being an important staple cereal of Asian countries, rice (*Oryza sativa* L.) contributes a major portion to human caloric intake and nutrition. Rice productivity is continuously challenged by various abiotic and biotic stresses which account for around 50% of global yield loss (Ishaq and Memon 2017). Among biotic stresses, brown planthopper (BPH, *Nilaparvata lugens* Stål) is a major devastating insect-pest of rice in South and Southeast Asia. It damages the rice crop by feeding phloem sap using its stylet-type

mouthparts (Normile 2008). In recent years, the BPH population has increased significantly due to the rapid adaptation of BPH-sensitive varieties which is further exacerbated by high humidity, optimum temperature, and excessive use of nitrogenous fertilisers beyond the approved doses (Sogawa 2015). Other factors contributing to BPH infestation are higher plant density and indiscriminate application of pesticides during the early development of the host (Rashid et al. 2017; Wang et al. 2008). Both nymphs and adults suck on cell saps of rice leaves, leading to dehydration of leaves, reduction in photosynthetic rate, leaf area, chlorophyll content, nitrogen level of leaf and stem and eventually death of plants resulting in 'hopper burn' (Cagampang et al. 1974). Other than the direct harm brought by BPH, it also causes indirect damage by transmitting viruses including rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) (Sogawa 1982; Cha et al. 2008; Cabauatan et al. 2009). There are four different biotypes in which BPH populations have been characterised (Khush et al. 1985). Of these, BPH biotype 4 is the most devastating and predominant in India. While different management strategies to control insect -pest damage, use of a host-plant resistance system is the most durable and environmentally safe approach for managing BPH (Brar et al. 2009; Kumar et al. 2020).

To date, 46 genes/QTLs have been designated from various cultivated and wild relatives of rice and assigned to the different chromosomes of rice. Of these, nine genes namely *Bph6*, *Bph9*, *Bph14*, *Bph17*, *Bph18*, *Bph26*, *Bph29*, *Bph30*, and *Bph32* (Guo et al. 2018; Zhao et al. 2016; Du et al. 2009; Liu et al. 2015; Ji et al. 2016; Tamura et al. 2014; Wang et al. 2015; Shi et al. 2021; Ren et al. 2016) have been cloned and characterised (Table 1). Among them, *Bph9*, *Bph14*, *Bph18*, *Bph26*, and *Bph30* encode a coiled-coil, nucleotide-binding site and leucine-rich repeat (CC-NBS-LRR) domain proteins. While *Bph17*, *Bph29*, and *Bph32* encode lectin receptor kinases (LRKs), B3 DNA-binding domain protein, and a unique short consensus repeat (SCR) domain protein, respectively. The CC-NBS-LRR proteins play central roles in perceiving the elicitors/effectors molecules and mounting the appropriate resistance response when infested by insects (Jacob et al. 2013). Wu et al. (2022) proposed that the *Bph6* protein enhances rice defense against BPH by regulating the accumulation of cell wall lignin. In another study conducted by Zheng et al. (2021), it was observed that the nymphs and adults of BPH feeding on NIL-Bph6 rice plants exhibited reduced weight gain and growth, indicating suppressed feeding by the BPH. The structure and functional analysis of *Bph14* revealed the induction of strong BPH resistance response by activating the salicylic acid (SA) pathway followed by the accumulation of numerous transcription factors (TFs) such as WRKY46 and WRKY72 leading to sieve tube blockage, which reduces the insect's feeding, growth, and survival (Hu et al. 2017). However, the molecular structure and functions of other BPH resistance genes are poorly known. Therefore, a precise understanding of the structure and function of BPH resistance genes is required to gain

Table 1 Details of cloned BPH R genes including their chromosomal location, characterised function, source and target biotype

Gene	Chromosome	Mapping region (kb)	Ids	Characteristics	Source	Target biotype	References
Bph6	4L	181.1	NCBI accession KX818197	Exocyst-localised protein	Swarnalata (Indica rice)	1, 2 and 3	Guo et al. (2018)
Bph9	12L	47	Accession no. KU216221	CC-NBS-NBS-LRR	Pokkali (Indica rice)	1, 2 and 3	Zhao et al. (2016)
Bph14	3L	34	Accession no. FJ941067	CC-NBS-NBS-LRR	*O. officinalis* (wild rice)	Mixed biotype from Hainan Province, China	Du et al. (2009)
Bph17	4S	79	Os04g0201900, Os04g0202350, Os04g0202500	OsLecRK1-OsLecRK4	Rathu Heenati (Indica rice)	1 and 2	Liu et al. (2015)
Bph18	12L	27	Accession no. KF890252	CC-NBS-NBS-LRR	*O. australiensis* (wild rice)	Korean	Ji et al. (2016)
Bph26	6S	135	Os12g0559400 and Os12g0559600	CC-NB-LRR	ADR52 (Indica rice)	1 and 2	Tamura et al. (2014)
bph29	6S	24	NCBI accession KC019172	B3 DNA binding protein	*O. rufipogon* (wild rice)	1, 2 and Bangladesh biotype	Wang et al. (2015)
Bph30	4S	22.4	Os04g08390	CC-NBS-NBS-LRR	*O. sativa* (Indica rice)	1, 2 and 3	Shi et al. (2021)
Bph32	6S	190	Os06g03240	SCR domain containing protein	Ptb33 (Indica rice)	Mixed biotype from Hainan Province, China	Ren et al. (2016)

deeper insight into the molecular mechanism underlying BPH resistance in rice.

The increasing disparity in connecting DNA sequences with protein structures presents significant challenges in understanding the function of proteins of interest. The current study emphasises on utilising comparative modelling for in silico prediction of 3-D structure of BPH resistant (R)/susceptible (S) proteins. Additionally, an interaction study is conducted to unravel the structural interactions between BPH-specific CC-NBS-LRR genes and other functional genes within the cell. Molecular profiling analysis and secondary structure prediction serve as crucial groundwork for exploring the evolutionary biology of plant- (R) genes. The obtained results contribute to a greater comprehension of the interplay between R protein-elicitor perception and plant defense signalling in response to rice planthoppers. Overall, these findings contribute to a better understanding of BPH resistance mechanisms and offer potential targets for developing effective strategies to combat this destructive rice pest.

Materials and methods

Sequence retrieval

The nucleotide and amino acid sequences of the successfully cloned BPH genes (namely *Bph6*, *Bph9*, *Bph14*, *Bph17*, *Bph18*, *Bph26*, *Bph29*, *Bph30*, and *Bph32*) were downloaded from National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) and Rice Genome Annotation Project (RGAP) database (http://rice.uga.edu/). The complete coding sequence and protein sequence were retrieved using the gene ids of the cloned BPH genes as given in the reported papers (Supplementary Table 1). The query nucleotide sequences were BLAST (Basic Local Alignment Search Tool) searched to identify the sequences in the other wild and cultivated species genomes submitted in RGAP website (http://rice.uga.edu/analyses_search_blast.shtml) and Ensembl Plants website (http://plants.ensembl.org/index.html). The BLAST search on RGAP and Ensembl revealed very low query coverage among various genomes of wild and cultivated rice. The homology alignment of query sequence was not totally aligned with the subject using default parameters (e-value threshold as1e-5). Similarly, BAC (Bacterial Artificial Chromosome) libraries of the rice genome were used to annotate the target genes. The genomic sequence retrieved from BAC was processed to determine the coding and non-coding regions. The protein sequences were retrieved in FASTA (fast-all) format and processed in Ensembl Plants for identifying target genes with chromosome location. The features of the gene were studied using various options available in the genome browser at the RGAP site. The sequence of methods followed for the present study has been represented in Supplementary Fig. 1.

Physicochemical characterisation of BPH R proteins

Various physical and chemical properties such as molecular weight, theoretical pI (isoelectric point), EI (extinction coefficient), AI (aliphatic index), II (instability index), +R and −R (total number of positive and negative residues) and GRAVY (grand average hydropathy) of cloned BPH R proteins were calculated using ExPASy's ProtParam web server tool (http://web.expasy.org/protparam/) (Gasteiger et al. 2005).

Phylogenetic analysis and motif identification

The Unrooted phylogenetic tree was constructed using the maximum likelihood method to analyse the evolutionary relationship among the cloned BPH R proteins of rice… All the protein sequences were imported in the MEGA (Molecular Evolutionary Genetic Analysis) software, version 10 (Kumar et al. 2018) (https://www.megasoftware.net/) and the reliability was checked with bootstrap replication value as 1000 and other default parameters remained same. The sequences were then aligned using the MUSCLE algorithm. The conserved motifs were identified using MEME (Multiple Em for Motif Elicitation) software, version 5.1.1 (Bailey et al. 2006).

Domain annotation

Domains from amino acid sequences of cloned BPH genes were extracted through a multi-source domain annotation server, MyCLADE. This server was used to annotate the query dataset with an available set of Pfam domains (http://www.lcqb.upmc.fr/myclade/index.php).

Model preparation

Swiss model

SWISS-MODEL is accessible via a web interface at http://swissmodel.expasy.org, or directly as a link from SWISS-PROT (Boeckmann et al. 2003) entries on the ExPASy server (Appel et al. 1994). The target sequence and template structure were identified and aligned for model building. A local pair-wise alignment of the target BPH sequence to the main template structures was calculated. The integrity of the models was analysed by C-score, giving an estimate of the variability of the template structures at this position in the result files. The parts of the model with no template information were assigned a *C*-score of 99.

Phyre^2

To predict and analyse the protein structure and function, Phyre^2 tool was used. Phyre^2 uses advanced remote homology detection methods to build 3D models, predict ligand binding sites and analyse the effect of amino-acid variants for the query protein sequences. With Phyre^2, function and mutations in the proteins were also predicted. After submitting the query protein sequence, 2° and 3° structures of the models, their domain composition and model quality were interpreted. The 3D structure of the successfully cloned BPH R genes was predicted by submitting the protein sequence of each gene individually (Fig. 1). The analysis included sequence analysis, 2° and disorder prediction, domain analysis and detailed template information. The information to the right of the image showed the structural template on which the top models were based, the confidence and coverage of the model with a link to interact with the 3D model using JSmol within the browser.

Prediction of protein interactions

For an overall understanding of cellular function, knowledge of all functional interactions between the expressed proteins is required, for which STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database was used (https://string-db.org/). The associations in STRING are included only if direct interactions (physical), as well as indirect interactions (functional) are specific and biologically meaningful (Szklarczyk et al. 2017). STRING showed a linkage of the query proteins with other functional proteins in the cell by collecting and reassessing available experimental data on protein–protein interactions, and importing known pathways and protein complexes from curated databases. For each protein–protein association stored in STRING, a confidence score scaled between zero and one was provided. The confidence score indicated the estimated likelihood that a given interaction is biologically meaningful, specific and reproducible, given the supporting

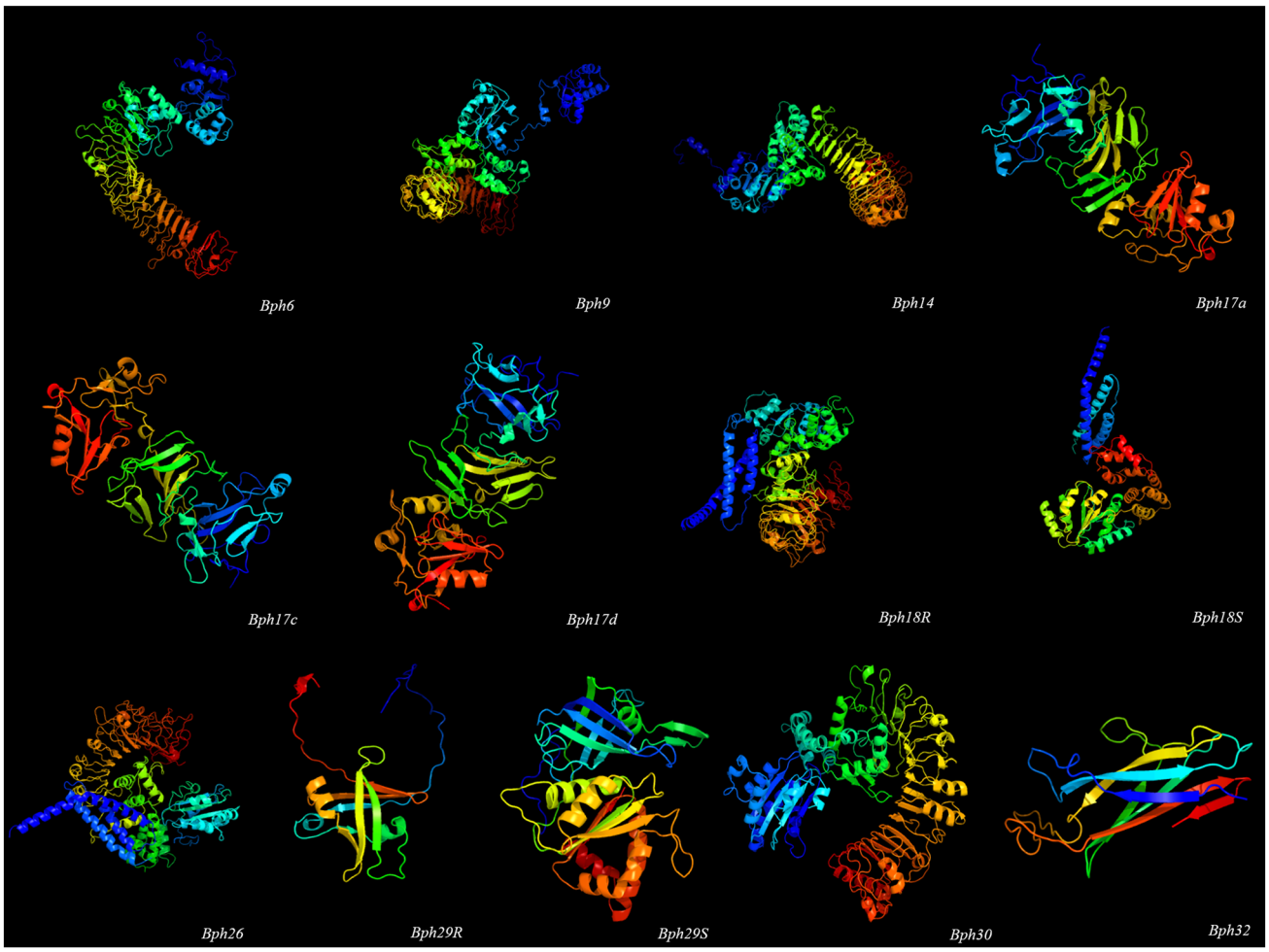

Fig. 1: 3D structure of the cloned BPH R proteins modelled by homology modelling server Swiss-model and Phyre^2 (The colours used in protein model depicts the different side chains from N to C terminal, following the rainbow colour pattern from red to blue)

evidence. Hence, the interacting units in STRING are the actual protein-coding gene loci (represented by their main, canonical protein isoform).

Preparation of ligands and protein docking

For docking, the 3D protein structure of BPH R genes and phytochemicals/ligands were downloaded from the NCBI and PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) in.sdf format. The sdf files were converted to.pdb format using Open Babel (O'Boyle et al. 2011). The energy of ligands as well as receptors for docking were minimised using Chimera 1.16 version tool. The ligands selected were jasmonic acid and salicylic acid with previous docking history (Gupta et al. 2019). All of the cloned BPH R proteins were docked individually with both ligands. AutoDock4 version (v4.2.6) (https://autodock.scripps.edu/) was used for docking with the work assisting tool python 3.10.0 (https://www.python.org/) to bind protein and ligand. The protein molecules were processed by adding hydrogen ions, merging non-polar hydrogen atoms, defining AD4 atom types, etc. (Fig. 2). Lamarckian Genetic Algorithm 4.2 was implemented and the receptor was kept rigid all throughout the study. The genetic algorithm was set for 100 runs with other parameters at default (250,000 energy evaluations). The docked models were analysed in UCSF Chimera 1.16 version tool (Pettersen et al. 2004) and were selected based on the appropriate interactive site and docking score. The docked files in pdb format were uploaded in PDBsum to know interacting residues (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) of phytochemicals [jasmonic acid and salicylic acid] with cloned BPH R proteins.

Results

Prediction of 3-D structure of BPH R/S proteins

Based on analysis of the targeted proteins, the domain composition of the BPH R and S proteins have indels leading to their different reaction towards BPH infestation. *Bph6* has deletion at 821 position in c4ecnA domain in LRR protein, *Bph9* and *Bph14* have deletions at 451 and 237 position respectively in c7jlvG domain in disease resistance protein roq1, *Bph17a*, *Bph17c* and *Bph17d* have deletions at 655, 623 and 646 positions respectively in c6xr4B domain

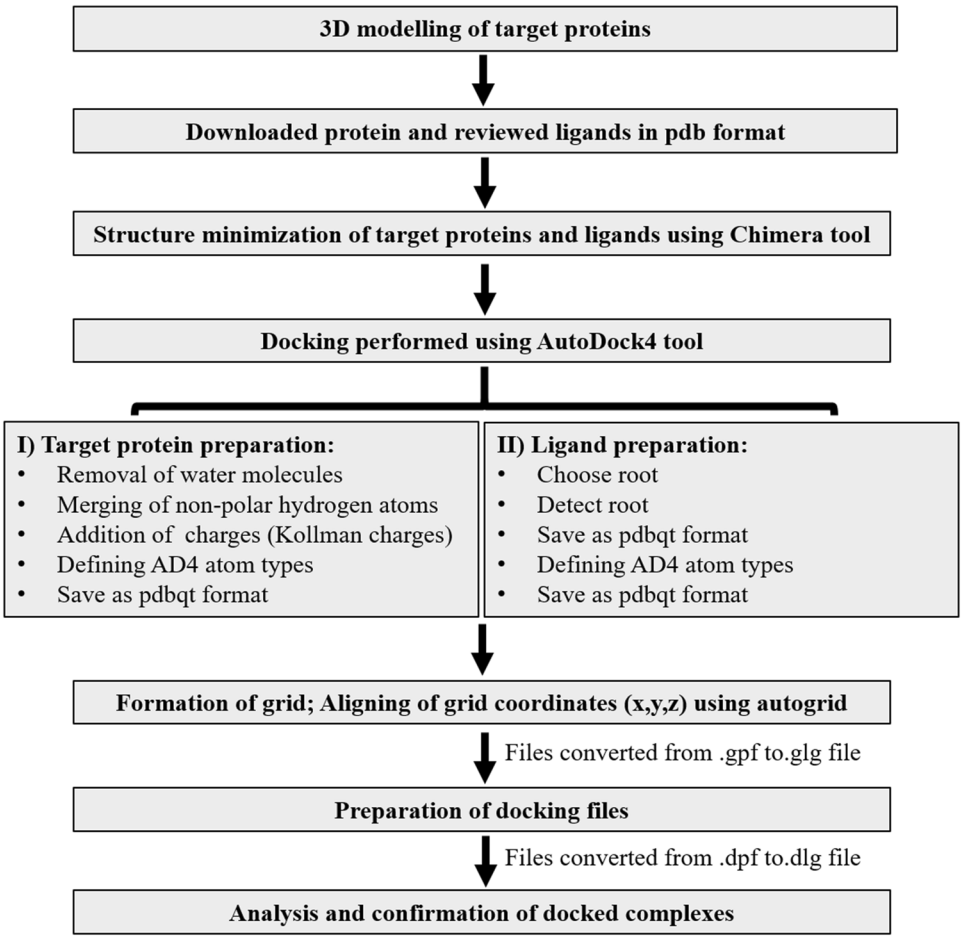

Fig. 2 Flowchart illustrating the sequential steps employed in docking of BPH R proteins

in LRR serine/threonine-protein kinase 2. The comparison of *Bph18* R and S proteins using SWISS-MODEL revealed truncation of protein leading to susceptibility against BPH in rice. Using UCSF Chimera, *Bph18* R domains (232–344 and 411–650) and *Bph18* S domains (233 to 329) were selected (Fig. 3a and b) and superimposed to visualise the truncation of *Bph18* protein in susceptible cultivar (Fig. 3c)where a deletion at 624 position in disease resistance rpp13-like protein4 was found to be associated with the susceptibility of *Bph18* (Fig. 3d). *Bph26* has deletion at 483 position in c4ecnA domain in LRR protein. These insertion-deletions and their respective positions help to target specific stretches of the gene or editing the DNA at particular locations. The analysis revealed that the majority of the proteins conferring resistance to BPH contained domains such as those found in disease resistance proteins like rpp13-like protein4, roq1, and LRR serine/threonine-protein kinase2. However, in two cases (*bph29* and *Bph32*) no resistance domain was found, still they are providing resistance against BPH (Wang et al. 2015; Zhao et al. 2016). *bph29* and *Bph32* may act as an enhancer or suppressor for other proteins or may give resistance due to phosphorylation or methylation as they are not directly related to the disease resistance NB-LRR family. The proteins were further explored with the help of STRING software to analyse the Interactome of the BPH R genes.

Physico-chemical properties

The molecular profile analysis revealed that CC-NBS-LRR domains containing BPH resistance genes have varying lengths of amino acid chains ranging from smallest (1082) for *Bph30* to longest (2024) for *Bph6*. The pI value of cloned BPH resistance genes ranged from 5.83–8.74, where *Bph6*, *Bph14*, *Bph30* with pI < 7 were found to be acidic and *Bph9*, *Bph18*, *Bph26* with pI > 7 indicated their basic nature. II of all proteins was above 40 suggesting that these are unstable proteins. The GRAVY index was also measured between −0.24 and −0.28 advocated that all proteins are hydrophilic in nature (Table 2). Since the GRAVY index values are negative, the proteins under consideration is considered as hydrophilic.

Phylogenetic and motif analysis

Neighbour-joining tree based on the protein sequence of cloned BPH resistance genes revealed two major groups (Supplementary Fig. 2). The *Bph30* (Biotype 1, 2 and 3) and *Bph6* (Biotype 1, 2 and 3) are clustered in group I. The most plausible reason is that they are rare atypical BPH resistance genes containing only LRR domains. The *Bph26* (Biotype 1 and 2), *Bph18* (Korean), *Bph9* (Biotype 1, 2 and 3), *Bph32* (mixed biotypes from China), and *Bph14* (mixed biotypes from China) are clustered in group II. The *Bph26* and *Bph18* showed high sequence similarity and both contain NB-ARC and LRR domains. The *Bph6* and *Bph30* contain two LRR domains. Whereas the remaining proteins have NB-ARC and LRR domains. The *Bph14* comprises a single NB-ARC domain while *Bph9*, *Bph18*, and *Bph26* comprise two NB-ARC domains. In addition to that the *Bph9*, *Bph18*, *Bph26* and *Bph32* contain other domains including AAA type ATPase domain. (Supplementary Table 2).

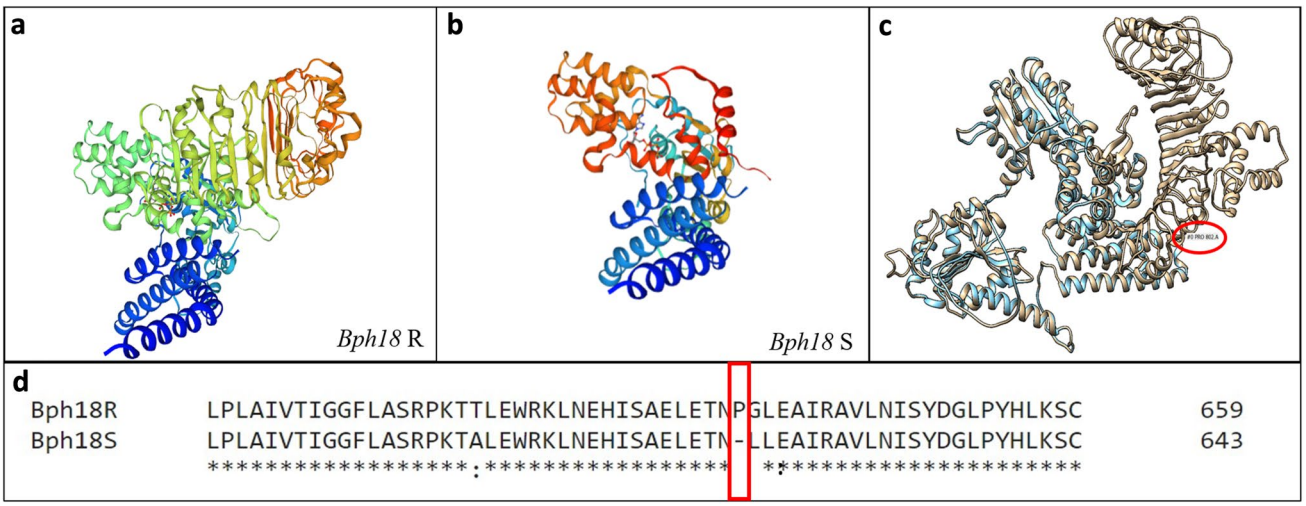

Fig. 3 **a** 3D structure of *Bph18* R domain; **b** 3D structure of *Bph18* S domain; **c** superimposed 3D model of *Bph18* R (represented in grey colour) and *Bph18* S (represented in blue colour) to visualise the truncation of *Bph18* protein in susceptible gene model., **d** sequence alignment of *Bph18* R and S depicting deletion of amino acid; Proline at 624th position in protein sequence of *Bph18* S

Table 2 Physiochemical characteristics of map-based cloned BPH resistance genes as predicted by ExPASy's ProtParam tool

Sr. no.	Characteristics	*Bph6*	*Bph9*	*Bph14*	*Bph18*	*Bph26*	*Bph30*
1	Sequence length	2024	1206	1323	1226	1218	1082
2	Molecular Weight	229,166.71	136,677.77	149,109.99	138,728.53	138,471.17	122,437.27
3	Isoelectric point	5.83	8.34	6.12	8.64	8.74	5.94
4	Total number of negatively charged residues (ASP + GLU)	264	159	176	156	156	135
5	Total number of positively charged residues (ARG + LYS)	221	167	160	171	172	113
6	Extinction coefficient	269,635	142,220	148,720	140,980	144,740	212,145
7	Instability index	49.50	42.38	48.99	41.43	42.14	55.78
8	Aliphatic index	90.18	98.13	93.70	98.35	96.76	87.61
9	GRAVY	−0.250	−0.282	−0.263	−0.247	−0.278	−0.274

Domain annotation of BPH R genes

Domain annotation of BPH R genes revealed that *Bph6* has LRR domain, *Bph9* has Rx N-terminal, RTC insert (RNA 3' terminal phosphate cyclase) domain, NB-ARC (ATPase) domain, and LRR domain, *Bph14* has Rx N-terminal, NB-ARC (ATPase) domain and LRR domain and FNIP repeat, *Bph17* has β lectin (D-mannose binding lectin), S locus glycoprotein, PAN (PAN-like domain) and Pkinase (Protein kinase domain), *Bph18* has Rx N-terminal, NB-ARC (ATPase) domain, LRR domain and FNIP repeat, *Bph26* has Rx N-terminal, NB-ARC (ATPase) domain and LRR domain, *Bph29* has B3 DNA binding, *Bph30* has AAA ATPase domain, LRR domain and FNIP repeat, *Bph32* has LEA (Late embryogenesis abundant protein).

Secondary structure prediction

The secondary structure of all the NBS-LRR domain BPH resistance proteins was predicted using SOPMA web servers. The result anticipated that all the proteins were cytoplasmic and soluble except *Bph30*. The *Bph30* is an endomembrane localised protein as it is localised in the endoplasmic reticulum, tonoplast, exocyst but not in the nucleus, Golgi apparatus, peroxisome or plastid. Based on the amino acid sequence, MINNOU server predicted this protein as endomembrane localized protein. The secondary structure prediction results revealed that there were high percentage of alpha helix, extended strands, and random coil accompanied by a small fraction of beta-turn in these proteins. The secondary structure composition of all the proteins is given in Supplementary Table 3. The *Bph6* contains alpha helix (34.66%), extended strand (16.41%), beta-turn (2.96%) and random coils (45.97%). The *Bph9* comprises a relatively higher alpha helix (52.16%), extended strand (12.35%), beta-turn (4.06%) and random coils (31.43%). Similarly, *Bph14* harbours alpha helix (56.99%), extended strand (7.71%), beta-turn (2.65%) and random coils (32.65%). The *Bph18* includes alpha helix (52.45%), extended strand (12.81%), beta-turn (3.67%) and random coils (31.08%). The *Bph26* encompasses alpha helix (52.71%), extended strand (12.97%), beta-turn (3.28%) and random coils (31.03%). However, the secondary structure composition of the *Bph30* was alpha helix (35.77%), extended strand (16.27%), beta-turn (3.60%), and random coils (44.36%). The analysis reveals that the LRR domains of *Bph6*, *Bph9*, *Bph14*, *Bph18*, *Bph26* and *Bph30* are mainly composed of Alpha helix conformation and hydrogen-bonded turn (Supplementary Fig. 3).

Interactome analysis of BPH R and S proteins

For each BPH R and S protein, the protein–protein interaction model was made in STRING (Fig. 4). The BPH R and S proteins along with their annotation and identity were explained forming clusters with the cellular functional proteins. In *Bph17*, G-type lectin s-receptor-like serine/threonine-protein kinase LecRK was found whereas disease related protein1 was found in *Bph9*, *Bph18* and *Bph26.* The interactome of the R and S genes showed that the BPH R genes interact individually whereas BPH S genes interact with other functional proteins like electron carrier proteins. The Os03g0848700 protein gives resistance against *Bph14* in rice and Powdery mildew resistance protein PM3b, putative, expressed in wheat (Table 3). It was observed that protein OsJ_28113 belonging to disease resistance NB-LRR family is encoded by *Bph9*, *Bph18* and *Bph26*. This protein has one Rx_N domain and two NB_ARC domains. The CC–NB–LRR protein of the NB–LRR family, is an immune receptor type similar to R proteins functioning in disease resistance. OsJ_28113 is encoded by three BPH resistance genes: *Bph9*, *Bph18* and *Bph26* derived from different plants, but reveal a similarity in the molecular mechanism

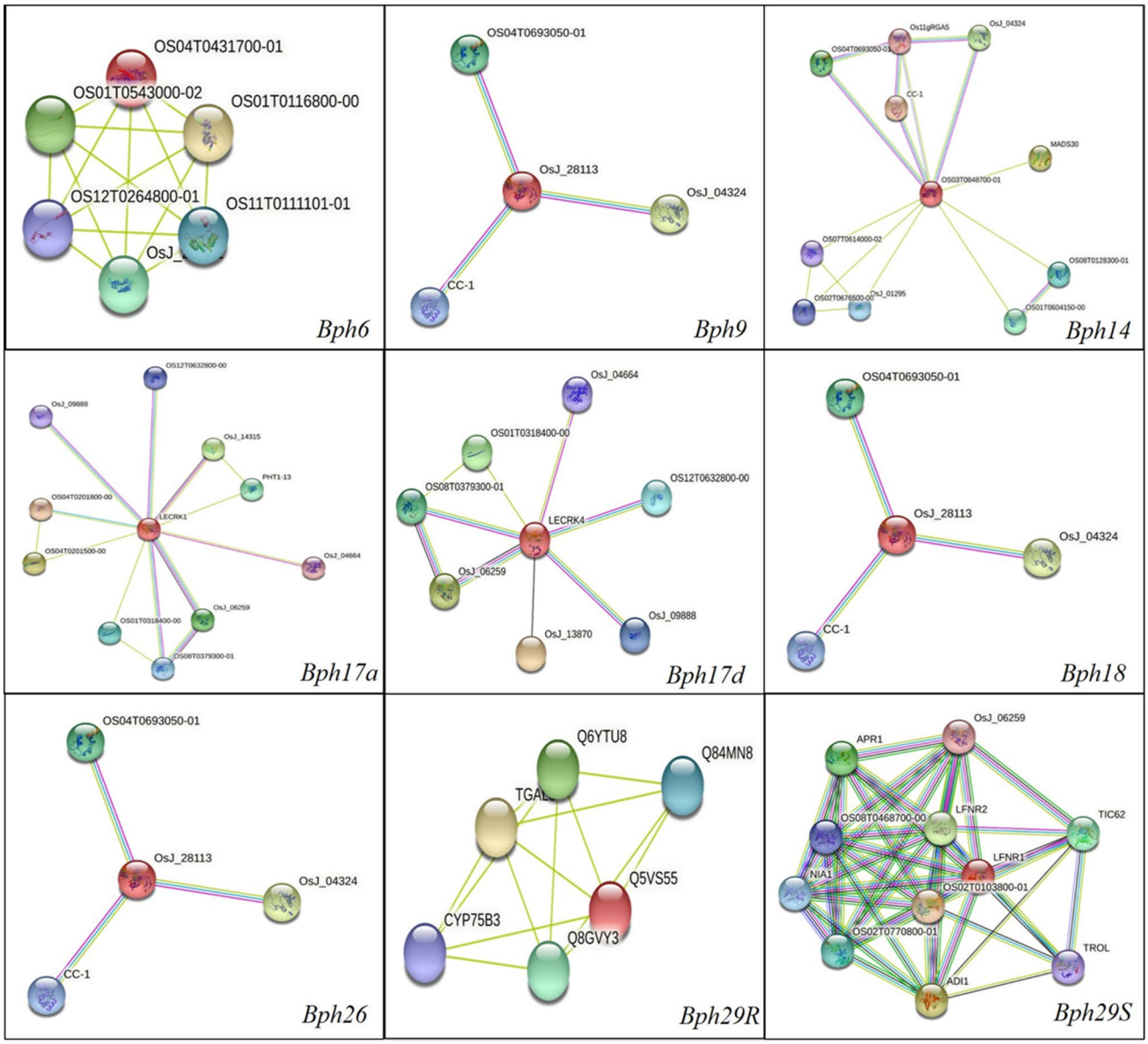

Fig. 4 Protein–protein interaction model of all BPH cloned genes using STRING software

for insect resistance. From the interactome, a model can be hypothesised that this protein is the master regulator having g-coupled receptors which transduce the extracellular signals into intracellular responses and activates the protein (Fig. 5). For disease management, we can aim three BPH R genes (*Bph9*, *Bph18* and *Bph26*) by targeting this particular protein (Fig. 6) to control vulnerability against BPH in rice. No interactions were found for *Bph32* with other proteins.

Molecular docking with phytochemicals

To find the best binding mode, Auto Dock was utilised for binding free-energy evaluation. Energy items calculated by Auto Dock comprise intermolecular energy, internal energy, torsional energy and unbound energy. Internal energy is composed of Vander Waals energy, hydrogen bonding energy, desolvation energy and electrostatic energy. The binding energy of *Bph6* with jasmonic acid and salicylic acid is 6.26 kcal/mol and − 4.8 kcal/mol respectively. *Bph9* has a binding energy of − 7.23 kcal/mol and − 6.49 kcal/mol with jasmonic acid and salicylic acid respectively. *Bph14* has a binding energy of − 4.17 kcal/mol and − 4.25 kcal/mol with jasmonic acid and salicylic acid respectively. *Bph17a*, *17c*, *17d* have a binding energy of − 5.45 kcal/mol and − 5.06 kcal/mol with jasmonic acid and salicylic acid respectively. *Bph18* has a binding energy of − 7.25 kcal/mol and − 6.65 kcal/mol with jasmonic acid and salicylic acid respectively. *Bph26* has

Table 3 List of BPH resistant (R) and susceptible (S) proteins along with their annotation and identity percent using STRING software

Protein	Gene	Annotation	Identity (%)
OS04T0431700-01	*Bph6*	Os04g0431700 protein	59.2
OsJ_28113	*Bph9*	Disease related protein 1; Os08g0539400 protein; Putative Pi-b protein	49.5
OS03T0848700-01	*Bph14*	Os03g0848700 protein; Powdery mildew resistance protein PM3b, putative, expressed	82.2
LECRK1	*Bph17a*	G-type lectin s-receptor-like serine/threonine-protein kinase lecrk1	100
LECRK3	*Bph17c*	G-type lectin S-receptor-like serine/threonine-protein kinase LECRK3	93.6
LECRK4	*Bph17d*	G-type lectin s-receptor-like serine/threonine-protein kinase lecrk4	100
OsJ_28113	*Bph18R*	Disease related protein 1; Os08g0539400 protein; Putative Pi-b protein	48.1
B8BMH8	*Bph18S*	Uncharacterized protein; Belongs to the disease resistance NB-LRR family	92.1
OsJ_28113	*Bph26*	Disease related protein 1; Os08g0539400 protein; Putative Pi-b protein	49.8
Q5VS55	*Bph29R*	B3 domain-containing protein Os06g0107800	100
LFNR1	*Bph29S*	Ferredoxin–NADP reductase, leaf isozyme 1, chloroplast; May play a key role in regulating the relative amounts of cyclic and non-cyclic electron flow to meet the demands of the plant for ATP and reducing power	100
A0A0P0WAN7	*Bph30*	Os04g0431700 protein	32.4
B8B1X3	*Bph32*	Uncharacterized protein	100

[a]Identity percentage describes the similarity of the query protein sequence with the conserved sequences present in the protein database. It signifies that the BPH R genes with conserved domains like NBS-LRR have high identity percentage in the reference genome

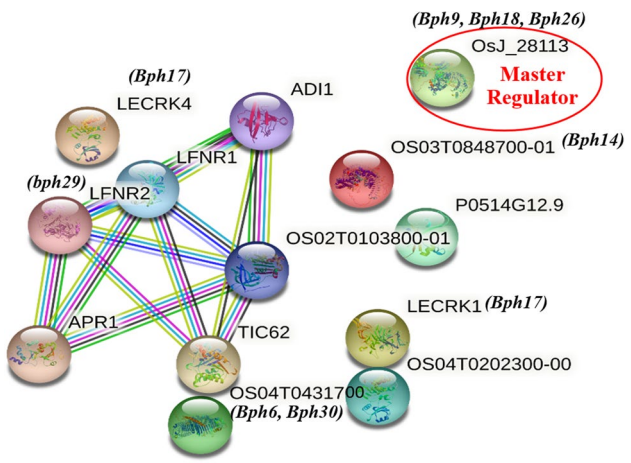

Fig. 5 Interactome depicting OsJ_28113 as the master regulator belonging to disease resistance NB-LRR family

a binding energy of − 6.9 kcal/mol and − 5.62 kcal/mol with jasmonic acid and salicylic acid respectively. *bph29* has a binding energy of − 5.04 kcal/mol with jasmonic acid and − 5.34 kcal/mol with salicylic acid. *Bph30* has a binding energy of − 4.72 kcal/mol and − 4.64 kcal/mol with jasmonic acid and salicylic acid, respectively. *Bph32* has binding energy of − 5.4 kcal/mol and − 4.75 kcal/mol with jasmonic acid and salicylic acid, respectively (Supplementary Fig. 4). Out of 9 docked complexes, the Auto dock binding energy of LRR domain of 7 complexes was highest with jasmonic acid indicating higher binding affinity of LRR domains towards jasmonic acid compared to salicylic acid (Table 4).

Discussion

Though great advancement has been made in predicting the experimental structure by X-ray crystallography and NMR, there remains a substantial disparity between the number of known proteins and their well-defined structures Therefore, the deployment of computational methods for protein structure prediction is urgently needed to bridge this 'structure knowledge gap'. In *O. sativa*, nine BPH resistant genes have been isolated using a map-based cloning approach. Among them, *Bph6*, *Bph9*, *Bph14*, *Bph18*, *Bph26* and *Bph30* encode CC-NBS-LRR domain proteins. The other three genes encode lectin receptor kinase, B3 DNA-binding domain, and a SCR domain protein suggesting a unique mechanism of resistance to BPH. Three of the CC-NBS-LRR protein encoding genes *Bph9*, *Bph18*, and *Bph26* are clustered on chromosome 12, while *Bph14* is located on chromosome 3. The CC-NBS-LRR regions are evolutionarily similar and largely found in clusters on plant genomes due to segmental and tandem duplication which generate closely related NBS genes (Meyers et al. 2003; Mchale et al. 2006; Leister 2004). Based on sequence analysis, the co-localization of two BPH resistance genes, *Bph18* (Ji et al. 2016) and *Bph26* (Tamura et al. 2014) within the genomic region of *Bph9*, suggested that they are functional allelic forms of *Bph9* (Zhao et al. 2016). A number of CC-NBS-LRR genes, despite their sequence similarity, showed amino acid substitutions or deletions in their NBS and LRR regions as documented by Ji et al. (2016). It has been suggested that the LRR domain of plant R proteins plays a crucial role in recognition of specific pathogen effectors and is involved in interaction with specific ligands which, in turn, elicit appropriate defense

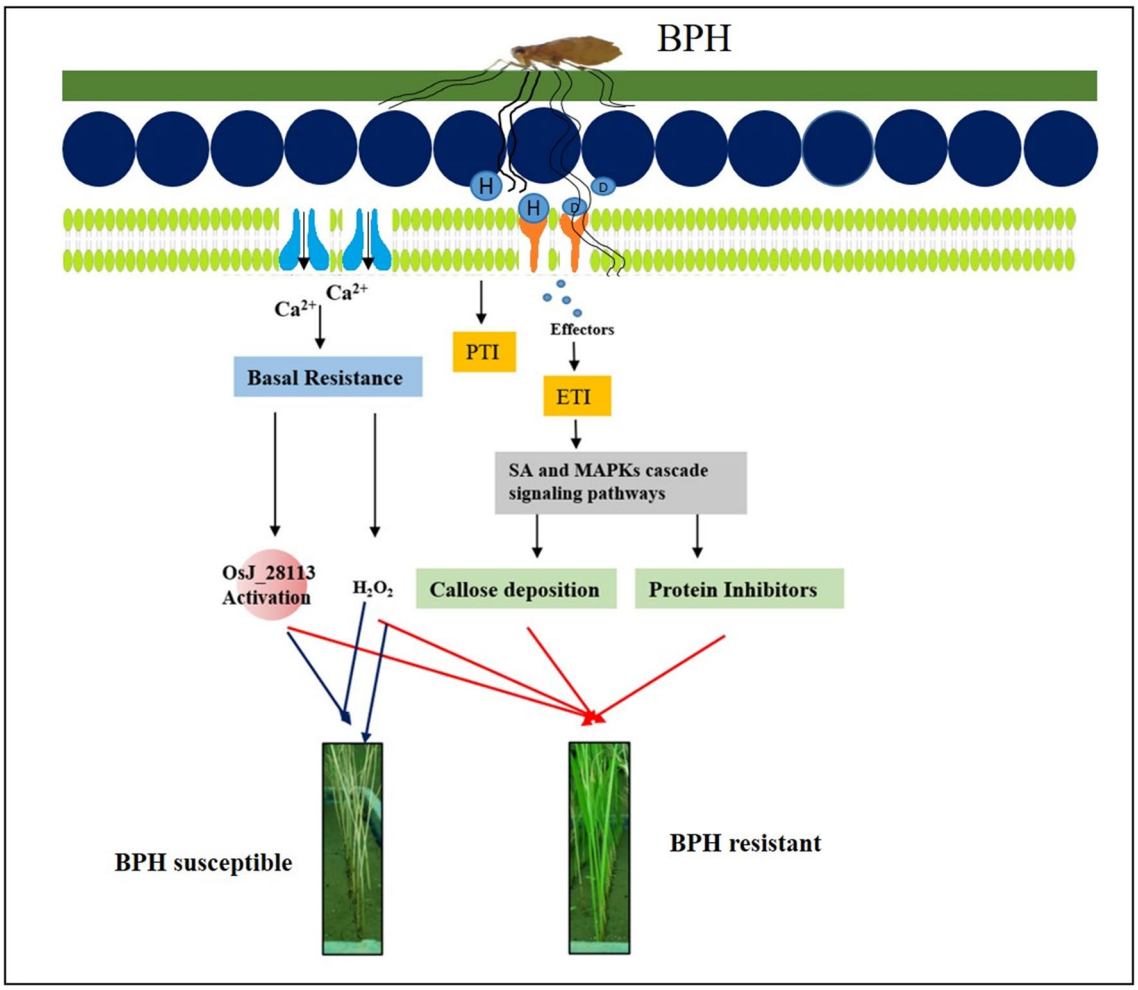

Fig. 6 Hypothesised model showing OsJ_28113 as the master regulator to target particular protein to control susceptibility against BPH in rice

responses (DeYoung and Innes 2006). This defense strategy of plants, to combat pathogens by creating gene clusters has led to the proliferation of R proteins under divergent selection during the co-evolution of plants and pathogens (Qian et al. 2017; Takken et al. 2006). Out of nine genes, six genes encode NBS-LRR domains. The role of CC-NB-LRR domain proteins is extensively studied in plant immunity. Recently, the R proteins have been reported in insect resistance against wheat hessian fly, etc. Therefore, our study primarily focuses on CC-NBS-LRR domain encoding genes. In this study, superimposition of the secondary structure of CC-NBS-LRR protein from resistant alleles with its susceptible counterpart showed InDels suggesting variations in resistance specificities of CC-NBS-LRR proteins to insect pests over time. Functional characterisation of different domains of CC-NBS-LRR protein has been performed for *Bph14* and *Bph9* (Hu et al. 2017; Wang et al. 2021). The molecular and functional analysis of CC-NBS1-NBS2-LRR domain of *Bph9* showed truncation of the LRR domain in the susceptible allele Bph^{99311}. The lack of LRR domain of susceptible alleles revealed loss of BPH resistance activity (Wang et al. 2021). Comparable outcomes were noted for *Bph18*, where the truncation of amino acids resulted in susceptibility.

A fully detailed view of all functionally relevant protein interactions of BPH-R and S proteins is essential to locate the molecular functions of individual proteins into their cellular context. The indirect associations such as genetic interactions or shared pathway memberships are equally important as physical interaction for a complete understanding of cellular function, allowing an effective design of experiments, such as site-directed mutagenesis, or the structure-based design of specific inhibitors. The interactome of the R and S genes indicated that in plants, the pathways' functioning relies on a series of reactions, with feedback inhibition playing a significant role. No discernible resistance domain was identified in the case of *bph29* and *Bph32* as *bph29* contains a highly conserved

Table 4 Corresponding binding energy (kcal/mol) scores and interacting residues of phytochemicals [jasmonic acid (JA) and salicylic acid (SA)] with cloned BPH R proteins

Gene	Docking score/binding energy (kcal/mol)		Ligand efficiency		Inhibition constant (uM)		Intermolecular energy (kcal/mol)		Electrostatic energy (kcal/mol)		Total internal energy (kcal/mol)		Torsional energy (kcal/mol)		Unbound energy (kcal/mol)		Interacting residues	
	JA	SA	JA	SA	JA	SA	JA	SA	JA	SA	JA	SA	JA	SA	JA	SA	JA	SA
Bph6	−6.26	−4.8	−0.42	−0.48	25.59	30.5	−8.05	−5.7	−1.11	−0.95	−0.47	−1.17	1.79	0.89	−0.47	−1.17	Pro 480, His 483, Trp 484, Leu 490, Pro 491, Ala 485, Pro 492	Pro 480, Pro 491, Leu 490, Trp 484, His 483
Bph9	−7.23	−6.49	−0.48	−0.65	5.0	17.53	−9.02	−7.38	−4.7	−4.36	−0.59	−1.04	1.79	0.89	−0.59	−1.04	Arg 709, Leu 1039, Leu 1061, Thr 1038, Ile 1086	His 958, Arg 853, Thr 959, Tyr 706, Lys 661
Bph14	−4.17	−4.25	−0.42	−0.42	882.25	773.08	−5.06	−5.14	−1.67	−3.3	−1.2	−1.01	0.89	0.89	−1.2	−1.01	Val 513, Phe 432, Tyr 421, Ser 517	Gly 210, Gly 212, Lys 213
Bph17	−5.45	−5.06	−0.36	−0.51	100.95	196.6	−7.24	−5.95	−1.06	−2.07	−0.44	−1.01	1.79	0.89	−0.44	−1.01	Glu 599, Met 601, Lys 579, His 578, Val 582, Phe 577, Thr 576	Met 782, Lys 788, Pro 784, Arg 783
Bph18	−7.25	−6.65	−0.48	−0.67	5.32	13.31	−8.99	−7.55	−1.49	−3.96	−0.63	−0.35	1.79	0.89	−0.63	−0.35	Leu 567, Leu 559, Pro 601, Gly 440, Gly 438, Leu 602, Leu 556, Leu 444, Thr 443, Phe 402, Ile 407	Lys 675, Thr 978, Tyr 886
Bph26	−6.9	−5.62	−0.46	−0.56	8.73	76.44	−8.69	−6.51	−2.85	−2.97	−0.53	−0.97	1.79	0.89	−0.53	−0.97	Phe 402, Thr 442, Thr 443, Pro 601, Gly 437, Leu 602	Met 53, Ser 11
bph29	−5.04	−5.34	−0.34	−0.53	201.79	122.18	−6.83	−6.23	−3.19	−2.89	−0.59	−0.69	1.79	0.89	−0.59	−0.69	Arg 138, Asp 136, Cys 127, Tyr 35, Glu 34	Tyr 35
Bph30	−4.72	−4.64	−0.31	−0.46	348.08	399.02	−6.51	−5.53	−1.55	−1.42	−0.54	−0.48	1.79	0.89	−0.54	−0.48	Lys 852, Arg 854, Pro 825, Pro 822, Leu 824, Ser 850	Pro 822, Pro 825
Bph32	−5.4	−4.75	−0.36	−0.47	109.73	328.41	−7.19	−5.65	−2.62	−2.87	−0.42	−0.56	1.79	0.89	−0.42	−0.56	Arg 150, Phe 149, Val 63, Lys 62, Gln 148, Tyr 151	Thr 70, Val 82, Pro 81

B3 DNA-binding domain (Punta et al. 2012), found exclusively in TFs that interacts with the major groove of DNA (Yamasaki et al. 2004). *Bph32* encodes an unknown protein that belongs to the complement control module/SCR domain family, a family of cell adhesion molecules (CAMs) that are considered to be types of lectin, or cell adhesion proteins (Parham 2005). The plant lectins have previously been reported to function as direct defence proteins to inhibit insect feeding (Michiels et al. 2010; Vandenborre et al. 2011) Therefore, it was hypothesised that these genes can be targeted individually by using their interactome and their interaction with other cellular proteins to provide resistance against BPH. Interactome analysis unveiled an additional finding that the genes controlling BPH resistance in rice might also play functional roles in other crops. This so-called 'interolog' transfer was based on the observation that orthologs of interacting proteins in one organism often exhibit interaction in another organism leading to the establishment of better orthology relationships (Walhout et al. 2000; Yu et al. 2004). It was observed that protein OsJ_28113 belonging to disease resistance NB-LRR family is encoded by three BPH-R genes: *Bph9*, *Bph18* and *Bph26* which can be targeted altogether for disease management. The ability to alter this information lays down the foundation for future functional genomic approaches (CRISPR- Clustered regularly interspaced short palindromic repeats, RNAi- RNA interference, Genetic engineering) to control susceptibility against BPH in rice.

utilising comparative modelling and molecular docking, the binding mechanisms of salicylic acid and jasmonic acid to the domains of BPH-R proteins were explored. The essential cellular processes, like gene regulation and signal transduction, rely on sequence-specific molecular recognition to direct proteins towards preferential interaction with specific nucleic acid or polypeptide ligands. The strength and specificity of such 'sequence recognition' play a crucial role in protein–ligand stability (Crocker et al. 2015; Farley et al. 2015; Tanay 2006; Rube et al. 2022). Protein–ligand binding free energy calculations are resultant of sequence-specific molecular interactions, salt bridges and hydrogen bonds interactions in the docking region along with the structural changes during complex unfolding (Fu et al. 2018) Moreover, the calculation of ligand binding affinities revealed that jasmonic acid dependent pathway promotes resistance against BPH with higher affinity than salicylic acid The variation in the hypervariable region of LRR repeats contributes to new ligand binding specificities for broader interaction efficiency. This research aids in advancing our knowledge of the complex mechanisms underlying the interaction of R proteins and ligands, indicating the co-evolutionary arms race between plant resistance and insect adaptation mechanism.

Conclusion

The findings from the in silico study lead to the conclusion that the adaptation of plant species to insect pest elicitors/effector molecules is a result of the ongoing variation in resistance genes as plants and insect pests continue to interact. To gain a deeper insight into the interaction between R genes and elicitors during BPH infection of rice planthoppers, and the activation of signalling cascades during this process, further molecular studies could be pursued. Moreover, a promising avenue for future research involves the development of BPH-resistant rice varieties by combining DNA-encoding BPH-R genes with phytochemicals. Such an approach holds potential for enhancing the plant's defense mechanisms against BPH infestations, paving the way for more effective pest control strategies in rice cultivation.

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Availability of data and materials All the data generated and analysed in this study are available in this article as supplementary material.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication Additional informed consent was obtained from all individual participants included in this study.

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