

INTEGRATIVE MOLECULAR APPROACHES TO PLANT DISEASE: A REVIEW

ABSTRACT: Modern molecular and bioinformatics technologies have made understanding host-pathogen interactions easier. Plants have many ways to protect themselves from microbial diseases, such as physical barriers, PAMP detection, and R genes that recognize pathogen-effector proteins and turn on effector-triggered immunity. Plant pathogen genome databases provide genomic and phenotypic data on plant pathogen species and information on plant-pathogen interactions. Map-based or positional gene cloning is improving our understanding of plant-pathogen interactions, with R genes being used to develop resistance to pathogens. Plant genomes typically contain several hundred nucleotide-binding site-leucine-rich repeats (NLRs), with their number, arrangement, and domain combinations varying by species. Bacterial blight (BB) severely impacts rice production, and about 37 of 44 resistance genes have been mapped and 15 cloned. Many disease-resistant wheat cultivars have been developed using powdery mildew leaf rust (Lr) resistance genes from wild relatives of *T. aestivum*. Over 140 genes are linked to powdery mildew resistance in *T. aestivum* and MutChromSeq have found new target genes. Cloning Arabidopsis resistance genes is essential for developing resistant cultivars and understanding R gene evolution. Some R genes encode proteins with nucleotide-binding site (NBS) motifs, and an LRR protects against *Erysiphe cruciferarum* powdery mildew. CRISPR/Cas9 gene editing is a major tool in plant genome editing, efficiently introducing target site mutations and improving plant immunity. High-throughput sequencing can identify and clone candidate resistance genes in different plant species, and gene editing technologies like CRISPR/Cas have illuminated site-specific mutagenesis and durable resistance.

Keywords: Defense, database, pathogen, resistance genes and clone.

1. INTRODUCTION:

Phytopathogens threaten livelihoods and societal growth by reducing crop yields and quality. Modern molecular and bioinformatics technologies make host-pathogen interactions easier to understand [1]. Scientists can analyze massive amounts of biological data by sequencing many pathogens and plant species using whole-genome sequencing [2]. “Plants have many ways to protect themselves from microbial diseases, such as physical barriers, PAMP detection, and R genes that recognize pathogen-effector proteins and turn on effector-triggered immunity” [3]. “While single R genes control qualitative resistance, multiple genes or quantitative trait loci control quantitative plant disease resistance” [4]. “Disease resistance is linked to the eight classes of nucleotide binding (NB) and leucine-rich repeat (LRR) domains in R proteins. CC-NBS-LRR (CNL) proteins, tobacco N, flax L6, and some RLK and RLP genes are resistance genes” [5, 6]. “*Arabidopsis thaliana*, *Oryza sativa*, *Gossypium sp.*, *Brassica napus*, and *B. rapa*; *Vitis vinifera*; *Triticum aestivum*; *Zea mays*; and *Hordeum vulgare*” [7, 8–18]. “Mega nucleases (MNs), zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas are needed to improve plant health” [19]. The CRISPR/Cas system is popular for its simplicity, low cost, efficiency, and reproducibility. CRISPR/Cas has been used to develop plant species' resistance to bacterial, fungal, and viral diseases [19].

2. Plant Pathogen Genome Databases

Genome databases study genetic diversity, disease, and host-pathogen interactions [20]. PhytoPath uses Ensembl genome portals to provide genomic and phenotypic data on plant pathogen species. The NIAS Genebank preserves and documents Japanese agricultural plant, microorganism, and animal genetic resources [21]. PathoPlant, a database of plant-pathogen interactions, explains signal transduction during plant pathogenesis. PHI-base contains fungal and bacterial pathogens' experimentally verified pathogenicity, virulence, and effector genes [22]. “Annotate, predict, and display host-pathogen interactions with HPIDB 3.0. Using integration and IMEx consortium curation protocols, Virus Mentha is a new resource for studying virus-virus and virus-host interactions. PCPPI is a large database for Penicillium-crop protein-protein interactions. Microorganism genome databases can amplify data, but plant pathogen genome databases are needed to understand disease resistance mechanisms” [23].

3. R genes and Plant NLRs

Map-based or positional gene cloning is improving our understanding of plant-pathogen interactions. Genetic map positions are used to isolate genes with known phenotypes and genomic locations. Hm1 from *Z. mays* was the first R gene cloned. developed resistance to *Chlioboluscarbonum's* HC toxin [8]. “Flax R genes include Cf-9, N, RPS2, RPM1 and L6. Advanced plant genomics and genetic engineering techniques make it easier to clone R genes from crops or their wild relatives and transfer them into elite breeding lines or cultivars” [24].

3.1.1 Plant NLRs

Nucleotide-binding site-leucine-rich repeats (NLRs) are made by different genes. They come in two main types: CNL (coiled-coil) and TNL (Toll/interleukin-1 receptor). Plants, animals, fungi, and protists have many NLR proteins. Plant genomes typically contain several hundred NLRs, with their number, arrangement, and domain combinations varying by species [25]. *Carica papaya*, *Cucumissativus*, and *Citrulluslanatus* have low NLR copy numbers. There is no correlation between genome size and gene count [26, 27, 28, 29, 30].

3.1.2 R Genes in *O. sativa*

Bacterial blight (BB) severely impacts rice production. About 37 of 44 BB resistance genes have been mapped and 15 cloned [7, 18, 31]. These genes are classified as receptor-like kinase (RLK), sugar will eventually be exported transporter (SWEET), executor genes (Xa10), Xa23 and other genes (Xa1 and Xa5). Over 100 R genes and 27 clones have been found in *Magnaportheoryzae*-caused rice blast. Pia protects against blast fungus *M. oryzae* with AVR-Pia [32–35]. Pi25, Pi36, Pi-64(t), and Pi-jnw1 from cultivar AS20-1 are blast resistance genes [4,12,36].

3.1.3 R Genes in *T. aestivum*

Breeding programs have developed disease-resistant wheat cultivars using powdery mildew leaf rust (Lr) resistance genes [91]. First resistance genes, Lr10, Lr21, and Lr1 were cloned in *T. aestivum*. Most of the 80 Lr genes characterized are from wild relatives of *T. aestivum*. Over 60 stem rust (Sr)-resistant genes have been found in wild relatives of *T. aestivum* [37–39]. *Blumeriagraminis* f. sp. *tritici* is a common disease of *T. aestivum* and causes severe yield loss. Over 140 T genes are linked to powdery mildew resistance in *T. aestivum*. Pm2 [14], Pm2a [15], Pm3 [40], Pm3b [41], Pm3c and Pm3b [42], Pm5e [17], Pm8 [13], Pm17 [43], Pm21 [16], Pm24 [44], Pm41 [45], Pm60 [46], PmR1 [47], and Pm2b [91] have been cloned using map-based cloning and sequencing. MutChromSeq has found new target genes. Target-sequence enrichment sequencing (TEnSeq) pipelines have cloned Pm genes, most of which contain an NLR, while resistance genes Pm38 and Pm46 encode ATP-binding cassette (ABC) and hexose transporters, respectively [48, 49].

3.1.4 R genes in *Z. mays*

“Fungal diseases threaten global maize production. Hm-1 was the first gene cloned against northern corn leaf blight and *Cochlioboluscarbonum*” [8]. A devastating fungal disease caused by *Setosphaeriaturcica*. A map-based cloning method identified and cloned four resistance genes: Ht1, Ht2, Ht3, and Htn1. Htn1 works against the most common NCLB races but depends on the maize genotype and environment. Only sixteen maize resistance genes have been cloned, with RppC being the most common. *Pucciniapolysora* causes southern corn rust (SCR), the most common disease in the US, Canada, Brazil, and China [108]. *Fusariumgraminearum* Gibberella stalk rot in maize is greatly improved by qRfg1, a major resistance quantitative trait locus. Map-based cloning was used to clone the CCT domain-containing gene ZmCCT. *Rhizoctoniasolani* banded leaf and sheath blight is resistant to ZmFBL41. MDR is a useful tool for developing durable resistance, but only one maize MDR gene (ZmMM1) has been cloned [51, 52]. “In maize-growing regions, Rab GDP dissociation inhibitor alpha (RabGDI α) is a host susceptibility factor for rice black-streaked dwarf virus infections. Sugarcane mosaic virus (SCMV) is a severe maize disease, and Scmv1 and Scmv2 provide complete resistance” [53, 54].

3.1.5 R Genes in *A. thaliana*

Cloning Arabidopsis resistance genes is essential for developing resistant cultivars and understanding R gene evolution. Most Arabidopsis R genes are TIR-NBS-LRR or LZ-NBS-LRR, with receptor-like kinases (RLKs) involved in plant development and defense [55, 56]. The TIR-NBS-LRR subclass has an N-terminal region that

resembles the cytoplasmic domain of Toll and interleukin-1 transmembrane receptors (TIRs), while the LZ subclass has a leucine zipper-like motif (LZ) [57, 58, 59, 60]. Some R genes encode proteins with nucleotide-binding site (NBS) motifs, and an LRR protects against *Erysiphecruciferarum* powdery mildew. Defense-signaling components EDS1, NDR1, and PBS1 are linked to RPP4-mediated resistance [61, 62]. “RFO, which is identical to WAKL22 in Arabidopsis, was characterized using map-based cloning. RPS5 is an NBS-LRR subclass, and cloning RPS5 genes has helped characterize two rps5-1 mutations that affect several R genes and confer resistance to both pathogens. In *Arabidopsis*, both subclasses confer resistance to *P. parasitica* and *P. syringae*, while RCY1 belongs to the CC-NB-LRR subclass and confers viral resistance” [63].

3.1.6 R genes in *S. lycopersicum*

The tomato genome has been extensively studied to understand resistance loci structure and organization. *S. lycopersicum* has over 100 loci, including the disease-resistant genes Pto, Ptil, and Fen. which resist *P. syringae* pv. *tomato* bacterial speck [64]. Cf-2 and Cf-9 from *Solanum pimpinellifolium* and Cf-4 and Cf-5 from *Solanum peruvianum* have been transferred into cultivated species to develop resistance to *Cladosporium fulvum*. Sw-5, introduced from the wild species *S. peruvianum*, developed resistance to TSWV, TCSV, and GRSV [60]. *Solanum* sp. has over 60 *P. infestans* resistance genes (Rpi genes). The first Rpi gene was Ph-1, followed by Ph-2 and Ph-2 in *S. pimpinellifolium* [65]. These genes created disease-resistant cultivars like *S. lycopersicum*, *S. pimpinellifolium*, *S. peruvianum*, *C. fulvum*, *F. oxysporum*, *G. rostrchiensis*, *M. javanica*, *P. syringae*, *V. dahliae*, TMV, TSWV, Ac-Ds, CC, NBS-LRR, and TIR.

3.2 NLR Annotation Tools

3.2.1 NLR-Parser

Java application NLR-Parser quickly annotates plant genome NLR complements. MAST output from translated amino acid sequences filters biologically curated motif compositions. The output shows the start and frame of the first NLR-specific motif, whether it is a TNL or CNL, and whether it is complete or fragmented. The NB-ARC domain sequence is useful for phylogenetics. NLR-parser searches for the complete set of NLR protein motifs to distinguish pseudogenes. Download the tool from Git-Hub [66].

3.2.2 NLR-Annotator

“NLR-Annotator annotates genomic sequence data at NLR loci as an extension of NLR-Parser. It splits genomic sequences into overlapping fragments and selects NLR loci with NLR-Parser. This method can find NLR loci candidates in stem rust, leaf rust, powdery mildew, and yellow rust resistance genes” [67].

3.2.3 NL Genome Sweeper

“The NB-ARC domain is used by NLGenome Sweeper to search genomes for NLR genes that protect against disease. It merges the NBS-LRR species' customized sequences into one fasta file. With high specificity for complete genes and structurally complete pseudogenes, the pipeline found 152 NBS-LRR proteins. 140 matches the manually annotated Arabidopsis NLR set with 96% sensitivity” [68].

3.2.4 NLRtracker

“InterProScan and predefined NLR motifs are used in NLRtracker to add annotations to proteome or transcriptome sequences and pull NLRs from the RefPlantNLR dataset based on core features. The NB-ARC domain is extracted for comparative phylogenetic analysis” [69].

4. DRAGO2

“DRAGO 2, an online platform for analyzing and predicting plant disease-resistance genes, accepts FASTA DNA or protein sequences. A Perl script predicts pathogen receptor genes (PRGs) and LRR, kinase, NBS, and TIR domains in PRGdb. DRAGO2 tools predicted over 1700 PRGs with the highest sensitivity” [70].

5. CRISPR Gene Editing

Its simplicity, flexibility, success rate, and cost-effectiveness have made the CRISPR/Cas9 system a major tool in plant genome editing. This system efficiently introduces target site mutations, including INDELS and base

substitutions [71] and other plant species have benefited from it. *A. thaliana*, *O. sativa*, *N. tabacum*, *S. bicolor*, *T. aestivum*, *Z. G. mays*, *S. lycopersicum*, *S. tuberosum*, *P. alba*, *M. domestica* and *Musa* species to fight viruses, fungi, and bacteria [72, 73,74]. The CRISPR/Cas9 system can be used to develop plant disease resistance by knocking out disease susceptibility genes (e.g., MLO), deleting or modifying cis-elements in promoters, modifying the amino acid sequence of surface receptor proteins to suppress secreted pathogen effectors, knocking down negative regulators of plant immunity, and modifying central regulators of the defense response. The system has developed O-resistant powdery mildew fungus varieties. *O. neolyopersici*, PMR4, *Erysiphe necator*, and *Plasmopara viticola* in *V. vinifera* [75, 76, 77, 78]. The CRISPR/Cas9 system provides primers for guide activity and target validation, as well as accurate sgRNA design tools. It designs sgRNAs in specific regions, checks for target specificity and genomic context, predicts target site specificity and sgRNA design for different CRISPR/Cas systems, provides on-target and off-target scoring and gRNA sequence analysis, and designs and constructs sgRNAs for CRISPR-Cas9-mediated genome editing [79]. AP2/ERF superfamily ethylene-responsive factors (ERFs) are essential for biotic stress adaptation. Fungus-resistant rice blast. *M. oryzae* was enhanced by a CRISPR/Cas9-mediated ERF922 gene mutation. AP2/ERF transcription factor knockdown reduced abscisic acid accumulation and increased *M. oryzae* resistance. Overexpressing defense genes is a key biotechnological tool for plant pathogen resistance [80, 81].

MORC proteins are essential nuclear regulators in prokaryotes and eukaryotes, silencing transcriptional genes and stabilizing genomes. MORC1 was found in *Arabidopsis* to protect against turnip crinkle virus (TCV), and AtMORC1, AtMORC2 and AtMORC6 are involved in multiple defenses against *P. syringae* and *Hyaloperonospora arabidopsidis*. The *Streptococcus pyogenes* CRISPR-Cas9 system was used to mutate the HvMORC1 and HvMORC6a genes in *H. vulgare*. Also studied were MORCs *S. tuberosum* (StMORC1), *Nicotianabenthhamiana* (NbMORC1) and SIMORC1 [82, 83]. The immune responses of various plants involve WRKY transcription factors. Mutant analyses in *Arabidopsis* have linked specific WRKY proteins to *P. syringae* defense responses. The susceptible variety (IR64) of *O. sativa* was resistant to the rice tungro streak spherical virus after a CRISPR/Cas9-mediated mutation in eIF4G. *Arabidopsis* and cucumber gained resistance to turnip mosaic virus and cucumber vein yellowing virus after recessive eIF4E gene mutations. Silencing the eIF4E gene has shown RNA virus resistance in *S. lycopersicum* and *C. melo* [84, 85].

Francisellanicida's FnCas9 (Cas endonucleases) could be a new way to attack the RNA virus genomes in plants. *N. benthamiana* and *Arabidopsis* were resistant to CMV and TMV using FnCas9 [86, 87]. The single-effector Cas13a protein of *Leptotrichia shahii* (LshCas13a) was a programmable RNA-guided single-stranded RNA (ssRNA) ribonuclease that protected the bacteria from bacteriophages [192]. "*O. sativa* was resistant to SRBSDV and RSMV using the LshCas13a system. The simple operation, good knockout effect, low cytotoxicity, high specificity, and universal applicability of the CRISPR/Cas system have made it more effective for disease resistance development by targeting the pathogen genome or host genes to interfere with susceptibility. Since CRISPR/Cas-induced mutations create pathogen-resistant genotypes when natural populations or wild relatives lack resistance resources, the system has garnered attention" [89]. "Intracellular NLR receptors recognize pathogen effectors and activate the immune system. The mechanisms of plant NLR activation are unknown, but animal NLRs oligomerize to activate downstream signaling after binding to their effectors. Some structural data has greatly improved our understanding of plant NLR activation. Genome editing advances will benefit sustainable agriculture" [86, 87, 90].

6. Conclusions

Plant immunity relies on NLRs to activate the strong resistance response that resists disease. Central nucleotide-binding (NB) and leucine-rich repeat (LRR) domains [91]. Understanding disease resistance is crucial, and high-throughput sequencing can identify and clone candidate resistance genes in different plant species. By pyramiding genes or altering plant and pathogen genomes, gene editing technologies like CRISPR/Cas have illuminated site-specific mutagenesis and durable resistance. Protein engineering has revolutionized NLR molecular recognition, and engineered intracellular immune receptors may ease resistance. NLR protein research is limited by the lack of three-dimensional structures and homology models. Modern computational technologies like AlphaFold and cryo-electron microscopy have illuminated NLR biological mechanisms and functional complexity. Designer NLR receptors may give crop plants broad-spectrum resistance using these technologies. Understanding protein structures, ligand binding, and host-pathogen interactions requires more tools.

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