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Review Article

Recent Understanding on Structure, Function and Evolution of Plant Disease Resistance Genes

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Plant disease resistance (*R*) gene triggers defense mechanisms by detecting non-self or modified self-molecules (*elicitors*) which results in hypersensitive response. Plant possesses a large number of *R*-genes with diverse recognition specificities which are activated in response to a variety of microbial pathogens. Many studies have shown that variation in *R*-genes is the result of gene duplication followed by divergence employing tandem or segmental duplication, recombination, unequal crossing over, transposable element activity, point mutation and diversifying selection. Large scale sequencing of plant species has revealed that most of the resistance genes are found in clusters of tandemly duplicated genes within the genome. Recent advances in genetics and genomics hold promise for better understanding of the mechanisms of *R*-gene evolution. The recent understanding on structure, function and evolution of disease resistance genes is reviewed with emphasis on the population dynamics of rice blast resistance gene *Pi54*. Based on evolution studies, novel strategies can be designed for developing durable disease resistance plant varieties.

Key Words: Disease Resistance Gene; Avirulence Gene; NBS-LRR; Evolution; Rice; *Arabidopsis*; *Pi54*

1. Introduction

The survivability of most organisms in varied environmental conditions depends on the presence of general resistance mechanisms, conditioned by inbuilt genetic system to maintain them. The antigenic variation in trypanosomes (Barry and McCullock 2001) and immunoglobulin gene formation in mammals (Blackwell and Alt 1989) are some of the classic examples of inherent resistance. Plants being sessile and only source of organic carbon, serve as food for almost all non-photosynthetic organisms on the earth. Plants are being constantly subjected to biotic and abiotic stresses. Their response to pathogen attack has been studied extensively and systematically. Diseased state of the plants is an outcome of three way interactions among pathogen, host and the environment, where every component of the interaction is presumed to be in favour of the pathogen. However, the mechanisms by which plant pathogens invade plant tissues and obtain nutrients are neither simple nor uniform. Nutrients are present in apoplasts or within the cell, therefore, access to these materials involve tissue and cellular degradation by the necrotrophic pathogens, whereas living host cells are manipulated by the biotrophic pathogens in such a way so that nutrients are obtained without killing the host cells (Faulkner and Robatzek 2012). The understanding of how plants recognize a foreign microbe as a pathogen and relay this information to the cellular system has practical applications for improvement and enhancement of agricultural production. Pathogens spread over long distances through direct and indirect modes of dispersal. Plant disease epidemics have severely affected humankind since the dawn of agriculture. An estimated amount

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of approx.15% of the crop produce is lost globally due to various diseases (Dangl *et al*. 2013)

A sophisticated multilayered immune system in plants bears testimony to co-evolution of pathogens and plants. This relationship between plant resistance and pathogen virulence through co-evolution was aptly described by *zig-zag* model (Jones and Dangl 2006). The initial reaction of host defense (*basal defense*) is based on the recognition of conserved pathogen derived molecules called pathogen/ microbe-associated molecular patterns (PAMPs/ MAMPs) by pathogen recognition receptors (PRRs) in the plasma membrane. PRRs typically recognize conserved microbial patterns and belong to the family of receptor like proteins (RLPs) or receptor like kinases (RLKs) (Beck *et al*. 2012). Involvement of PAMPs in generation of plant defense response is demonstrated by a well studied model of PAMP triggered immunity in plants based on the recognition of bacterial flagellin, flg22 through *Arabidopsis* FLAGELLIN SENSING 2 (*FLS2*) (Boller and Felix 2009). Upon ligand identification, FLS 2 forms a complex with the LRR-receptor kinase BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (BAK1) that induces production of reactive oxygen species (ROS), activation of calcium-dependent and mitogen activated protein kinases (CDPKs or MAPKs) (Boller and Felix 2009).

Specialized plant pathogens can evade or suppress this MAMP triggered immunity (MTI) by secretion of virulence factors called *effectors*. A subset of these effectors, referred to as avirulence factors (AVRs), which can be recognized by the resistance proteins that trigger a second layer of host defense, referred to as effector triggered immunity (ETI) or *R*-gene mediated defense, characterized by a rapid, localized cell death at the infection site, referred as *hypersensitive response* (HR). The HR leads to the signaling of distal plant organs to activate defense response genes which provide protection against subsequent infection by the pathogen. The selective pressure on potential pathogens leads to development of genetic variants that are no longer recognized by the receptors, leading to compatible interaction *i.e*. susceptibility. The specific demonstration of evolutionary battle between plants and pathogens has been exemplified by the interaction between tomato and the soil-borne fungus, *Fusarium oxysporum* (Houterman *et al*. 2008). This fungus employs effector protein AVR3 to suppress MTI that can be recognized by tomato R-protein I-3 which subsequently trigger ETI. To counteract this, the fungus produces or has evolved to produce a second effector (AVR1) that suppresses I-3 mediated defense. Finally to prevent successful infection by the pathogen, the host plant has evolved to produce R-protein I-1 that recognizes AVR1 to re-activate host defense mechanism (Houterman *et al*. 2008).

The pioneering work on genetics of plant disease resistance involving HR was done by Harold H. Flor in linseed – *Melampsora lini* system (Flor 1956). Flor proposed a *gene-for-gene* hypothesis that classically demonstrated genetic interaction between plant and pathogen. This hypothesis states that for every resistance (*R*) gene in a plant, there is a corresponding avirulence gene (*Avr*) in the pathogen. The interaction between the two corresponding genes *i.e*. the host resistance gene and the pathogen avirulence gene leads to incompatibility (resistance). This model hypothesizes that there could be direct or indirect physical interaction between a ligand produced by the pathogen with a corresponding plant receptor and ultimately triggers activation of downstream defense response genes.

Martin *et al*. (1993) provided first evidence of direct interaction of tomato *Pto* gene with *avrPto* from *Pseudomonas syringae* pv. *tomato*. Apart from direct interaction, evidences indicate that R proteins also act as *guard* of a specific component of the basic defense pathway (Guard hypothesis). If that component is modified by a pathogen effector molecule, the modification is recognized by the R protein and defense response is activated (Van Der Biezen and Jones 1998). In *Arabidopsis*, modification (phosphorylation or cleavage) of RIN4 protein by *Pseudomonas* type III effector molecules leads to activation of *R*-gene *RPM1* (Mackey *et al*. 2004). Recent understanding about the action of *R* genes in plants indicates that different *R* genes feed into a

common signaling pathway. In *Arabidopsis*, mutation in NDR1 prevents activation of defense responses *via* two proteins RPM1 and RPS2 that guard RIN4 component of the basic defense mechanism against bacterial pathogen *P. syringae*. The NDR1 protein spans the plasma membrane and interacts with RIN4, but the mechanism by which it receives signals from the R-proteins and transmits them into a signaling pathway is not yet clear.

Disease resistance genes have been extensively reviewed earlier (Bent 1996, Ellis and Jones 1998, Martin 1999, Michelmore 2000, Ellis *et al*. 2000, Jones 2001, Meyers *et al*. 2005) underpinning its importance in sustainable agriculture. The present review summarizes the current understanding about the structure, function and evolution of plant disease resistance gene and how deluge of genomic resources can be utilized for durable resistance breeding programme.

2. Classes of Plant Disease Resistance Genes Based on Structural Features

Numerous *R*-genes identified, cloned and characterized in different plants have been categorized in eight classes (Table 1) based on their amino acid motif organization (Gururani *et al*. 2012). The Leucine Rich Repeats (LRRs) domain is present in majority of the R proteins and is implicated to play an important role in recognition and specificity (Fig. 1). However, the first disease resistance gene

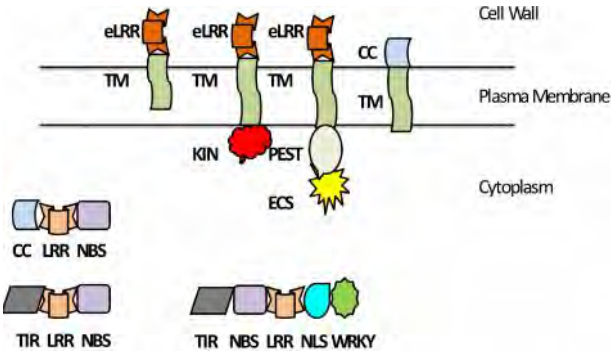

Fig. 1: Different domains of plant disease resistance proteins. LRR: Leucine Rich Repeat, eLRR: Extracellular LRR, NBS: Nucleotide Binding Site, CC: Coiled Coil, TIR: Toll-Interleukin Receptor, NLS: Nuclear Localization Signal, WRKY: DNA binding Domain (60 amino acids), TM: Trans-membrane Domain, KIN: Kinase Domain, PEST: Degradation Domain (proline, glycine, serine, threonine), ECS: Endocytosis Cell Signalling Domain

to be cloned and characterized was *Hm1* in maize (Johal and Briggs 1992). *Hm1* provides resistance to the maize fungal pathogen, *Cochliobolus carbonum*, by inactivating the HC toxin produced by this fungus. *Hm1* represents a class of resistance genes that encode *detoxifying enzyme*. Second major class of *R*-genes include the genes encoding for cytoplasmic proteins with a nucleotide binding site (NBS), a C terminal leucine rich repeat (LRR) and coiled-coil (CC) domain at the N-terminus. *Arabidopsis RPM1* and *RPS2* and tomato *I2* resistance gene belongs to this class. The third class of resistance genes consists of cytoplasmic protein possessing NBS and LRR motifs

Table 1: Structure and classes of plant disease resistance genes

Class	Structure	R Gene	Location	Plant
1	Toxin Reductase	*Hm1*	Cytoplasm	Maize
2	NBS-LRR-CC	*RPM1, RPS2, I2*	Cytoplasm	*Arabidopsis*, Rice, Tomato
3	NBS-LRR-TIR	*N, L6, RPP5*	Cytoplasm	Tobacco, Flax
4	eLRR-TM	*Cf-2, Cf-4, Cf-9, FLS2*	Transmembrane	Tomato, *Arabidopsis*
5	eLRR-TM-KIN	*Xa21*	Transmembrane	Rice
6	eLRR-PEST-ECS	*Ve1, Ve2*	Transmembrane	Tomato
7	TM-CC	*RPW8*	Transmembrane	*Arabidopsis*
8	TIR-NBS-LRR-NLS-WRKY	*RRS1-R*	Cytoplasm	*Arabidopsis*

and an N terminal domain with homology to the mammalian toll-interleukin-1 receptor (TIR) domain. The tobacco *N* gene, flax *L6* gene and *RPP5* gene are categorized under this class. The fourth major class of resistance gene family devoid of NBS motif consists of extra cytoplasmic leucine rich repeats (eLRR), attached to a trans-membrane (TM) domain. The eLRR are known to play an important role in activation of defense proteins like polygalactouronase inhibiting protein (PGIPs), even though they are not directly involved in pathogen recognition and activation of defense genes. The *Cladosporium fulvum* resistance genes (*Cf-2, Cf-4, Cf-9*) of tomato and *Arabidopsis FLS2* are some examples of this class. The fifth major class comprises of eLRR, a transmembrane (TM), and a (serine-threonine) kinase domain (KIN). In rice, *Xa21* gene that provides resistance to *Xanthomonas oryzae* pv. *oryzae* represent this class. The sixth class contains those genes which have putative extracellular LRR, along with a PEST (Pro-Glu-Ser-Thr) domain for protein degradation and short protein motifs for receptor mediated endocytosis (*e.g*. tomato *Ve1* and *Ve2* genes). The *Arabidopsis* RPW8 protein is an example of seventh major class of resistance genes containing trans-membrane (TM) protein domain, fused to the coiled-coil (CC) domain. The 8th class includes the *Arabidopsis RRS1 R*-gene which consists of putative nuclear localization signal (NLS) and a WRKY domain, besides TIR-NBS-LRR domains. The WRKY domain is a 60 amino acid region defined by consensus amino acid residues WRKYGQK at its N-terminal end, along with novel Zinc finger like motif. The list of disease resistance genes family is likely to increase with the increasing availability of genome sequencing data of different plant species. Most of the disease resistance genes exhibit dominant inheritance, although recessive inheritance is also common. Some of the *R*-genes exhibiting recessive inheritance includes barley *mlo*, rice *xa5* and *xa13* and *Arabidopsis rrs1 R*-gene.

3. Functions of Resistance Genes

Studies on the understanding of resistance gene function and its downstream signaling mechanisms should involve the confluence of high-throughput omics, molecular biology, genetics and plant breeding approaches. However, knowledge of these fields is yet to be converged to delineate common mechanisms guiding functioning of *R* gene(s) in plants. Recent understandings implicate the role of mitogen-activated protein kinase (MAPK) cascades, ubiquitin and E3, and E2 as signaling module downstream of receptor that transduce the signal. MAPK cascades are involved in signaling multiple defense responses like synthesis and signaling of plant stress hormones, generation of reactive oxygen species (ROS), ethylene biosynthesis, defense gene activation leading to phytoalexin biosynthesis, cell wall strengthening by the deposition of callose and hypersensitive response (Dixon 2001, Greenberg and Yao 2004, Ausubel 2005, Glazebrook 2005, Jones and Dangl 2006, Volt *et al*. 2009, Boller and Felix 2009, Coll *et al*. 2011, Spoel and Dong 2012, Meng and Zhang 2013). Ubiquitination play pivotal role in cell signaling that regulates several processes including protein degradation and immunological response utilizing functional proteasomes and protein targeting (Marino *et al*. 2012).

4. Organization of Resistance Genes in the Genome

The unveiling of complete genome sequence of the model plants like *Arabidopsis* and rice has been helpful in genome wide identification of disease resistance genes. In *Arabidopsis* genome, 149 NBS-LRR genes have been reported (The Arabidopsis Genome Initiative 2000). Annotation of genic sequences indicated that TIR-NBS-LRR (TNL) class predominates in the genome than the CC-NBS-LRR (CNL) class. TNLs are more homogeneous and have been amplified more recently. Sequence comparisons among sub-groups of *Arabidopsis* NBS-LRRs demonstrated wide diversity in the LRR sequences and this might have resulted due to the selective pressure of the pathogens. The completion of International Rice Genome Sequencing Project (IRGSP) has also revealed that the rice chromosomes 11 and 12 have 289 R-like and 28 defense response-like genes (The Rice Chromosomes 11 and 12 Sequencing Consortia 2005). Rice chromosome 11 has several large clusters of fast evolving disease

resistance genes that originated by tandem duplication and subsequent divergence under the selective pressure of rice pathogens. However, unlike *Arabidopsis*, most of the NBS-LRR class belongs to CNLs. Genomic analyses have also identified that TIR (Toll Interleukin receptor) proteins are abundant in both the genomes. Two new *R*-gene families *viz*. TIR-X (TX) lack both the NBS and LRR domains whereas TIR-NBS (TN) lacks only the LRR domain.. Most of the proteins are extremely well conserved in *Arabidopsis* and rice, suggesting that both belong to the ancient protein families.

Genes conferring race specific resistance in a typical gene-for-gene manner are commonly clustered in the host genome. Molecular dissection demonstrated that this clustering resulted from tandem duplications of paralogous sequences. A cluster of *R*-genes (rp1 complex) on the short arm of chromosome 10 of maize that confer resistance to *Puccinia sorghi* is one of the most common examples of tandem duplications (Saxena and Hooker 1968). Within the *rp1* complex, fourteen dominant genes have been identified which together provide a biotype specific resistance to maize rust pathogen. In addition to *rp1* complex, two other loci *rp5* and *rpG*, mapped to approximately. 2 to 3 cM distal to the *rp1* complex have also been identified. In flax, five loci *viz*. K, L, M, N and P have been identified that provide resistance to different races of *Melampsora lini* (Jones *et al*. 1997).

Some members of a resistance gene family are often arranged as tandem direct repeats, which is in consistent with their origin through gene duplication and their continued evolution through unequal chromosomal exchange. The *Xa21* gene family in rice located on chromosome 11 has evolved by transposition, recombination and duplication (Ronald *et al*. 1992; Song *et al*. 1997). Seven *Xa21* family members (A1, A2, B (Xa21), C, D, E and F) have evolved differently in response to pathogen attack (Song *et al*. 1995; Wang *et al*. 1995). Qu *et al*. (2006) also reported six paralogs in the *Pi-9* locus (*Nbs1* to *Nbs6*) arranged as tandem repeats in a 76 kb genomic region having high homology to the NBS-LRRs. The clustering of homologs at a locus provides possibilities for recombination to evolve new specificities of resistance when the corresponding *Avr* gene in pathogen gets mutated. A cluster of eleven tandemly duplicated defense response genes has been reported in a rice sheath blight resistance QTL qSBR11-1 (Channamallikarjuna *et al*., 2010).

Comparative mapping studies have shown a remarkable synteny among grass genomes (Devos and Gale 1997). Because of the observed synteny among the cereal genomes, it has been proposed that the grasses could be used as a single genetic system. Resistance genes and their analogues, however, may not always syntenize. The barley *mlo* and *Rpg*1 genes are not found in the syntenic region in the rice genome although the order of the flanking markers is conserved between barley and rice. In addition to disease resistance genes with known specificities, resistance-like gene sequences (RLGs) whose functions are unknown, map as clusters in rice, *Arabidopsis*, potato, tomato and soybean genomes. Based on comparative mapping studies on monocot RLGs, Leister et al (1998) reported that resistance genes diverge more rapidly than the rest of the genome through sequence divergence and ectopic recombination. For example, out of six rice and seven barley RLGs tested only ten maps to the syntenous regions on the foxtail genome.

R genes have remarkable property of rapid diversification under selective pressure from the pathogens. Most plant species contain a large number of highly polymorphic disease resistance genes having common structural domains (Ellis and Jones 1998). The DNA rearrangements have been advocated to play a crucial role in *R*-gene evolution allowing plants to generate novel resistance specificities to match the changing virulence pattern of the pathogen. This hypothesis has been supported by the study of maize rust resistance locus, *rp1*. Recombination of flanking markers in *rp* locus is associated with the creation of novel resistance phenotypes (Richter *et al*. 1995).

5. Role of Chromosomal Duplication and Recombination in Resistance Gene Evolution

Duplication of chromosomal segments during evolution plays a pivotal role in creating complex

genetic systems (Ohno 1970). Many new loci, altered gene family number through recombination or generation of repeated sequences within a gene are the outcomes of duplication. For example, human and mouse genome contain Major Histocompatibility Complex (MHC) that emerged as a result of chromosomal duplication. Similarly, in case of disease resistance genes in plants at least two clusters of *Cf* 9 resistance gene homologues have been identified on short arm of chromosome 1 of tomato, suggesting that *Cf* clusters are the products of duplications (Jones *et al*. 1997).

Similarly, genic recombination events can result in amplification and reduction of the number of resistance gene family members. As reported in case of tomato-*Cladosporium fulvum*, the presence of two functional *Cf*2 genes might be the result of a recent gene duplication event (Dixon et al. 1996). Analysis of the *Cf2/Cf5* locus, where only a few sequence homologous to *Cf* gene reside, has revealed a rare susceptible recombinant that is the result of an unequal crossover event leading to a reduction in the number of *Cf* homologues (Ellis et al. 1995). Molecular analyses of the five *Cf4/Cf9* disease sensitive recombinants demonstrated that chromosomal mis-pairing of intergenic sequences and unequal crossing over might have generated these recombinants.

The evidences of recombination and duplication have also been reported in several other resistance gene families. A large duplication of at least 17 kb genomic region has been reported in the *Xa21* multigene family. One of this duplicated genes confer the same race specific resistance similar to the *Xa21* gene (Song *et al*. 1997). Similarly, duplication and diversification of the *Pto* gene family has generated alternate recognition capabilities of the encoded proteins. The *M* locus of flax carrying tandemly arrayed specificities might have evolved from an ancestral *M* gene by a rare duplication event. Repetitive DNA flanking to this locus might have enhanced subsequent duplication through unequal crossing over events (Anderson *et al*. 1997).

In addition to swapping of large gene regions, recombination can lead to fine structural changes within a gene by recombination. The repeated structure of LRR coding region could facilitate intra- and intergenic recombination leading to expansion and contraction of the LRR numbers as demonstrated in the mutants of *M* and *Rpp5* genes. For instance, the wild type *M* gene contains two DNA repeats encoding LRRs. However, spontaneous mutants identified in the *M* gene contain a single LRR region. The mutant alleles with a single LRR repeat might have been generated by an unequal exchange between the first repeat in one *M* gene and the second repeat in its homologue. A fast neutron generated *Rpp5* mutant contains an intragenic duplication of four complete LRRs. This duplication might have arisen from an unequal crossing over event between two identical sequences in the LRRs (Parker *et al*. 1997).

Rapid sequence exchange among tandemly repeated gene families generally lead to sequence homogenization between constituent members of the gene family, though variability is maintained during the course of evolution. Parniske *et al*. (1997) sequenced three haplotypes at the *Cf4/Cf9* locus and comparisons of intergenic regions revealed a high degree of sequence rearrangements, and similarities in the coding regions. The similarities between conserved regions could be the result of gene conversion events or from successive rounds of reciprocal recombination. For instance, the meiotic stability of *Cf9* gene in a homozygous background is dramatically reduced in a *Cf4/Cf9* trans heterozygous background. It has also been proposed that the polymorphism of the intergenic regions suppresses unequal recombination in the homozygotes and sister chromatids, thereby preventing sequence homogenization of the gene family. In this situation, recombination within a coding region may actually contribute to the maintenance of a useful combination of resistance gene specificities. In a *Cf4/Cf9* trans-heterozygous background, homologous sequences aligned unequally are used as recombination templates. Such unequal recombination alters the number of gene family members as well as the composition of the clusters resulting in increased variation within the population. This has been demonstrated in case of rice blast resistance gene, *Pi54*, where a single point mutation in the regulatory

region has been attributed to be the cause of the resulting susceptible phenotype (Sharma *et al*. 2005).

Admittedly, the new race specificities arise due to recombination either by reshuffling of existing genes or by creation of novel resistances to those biotypes for which there were no resistance. Wilkinson and Hooker (1968) reported that thirteen variants identified at the *rp1* complex of maize had a resistance spectrum different from the parental lines from which they were derived. Of these, eight variants retained a subset of biotype specificities of one of their parents, suggesting that these variants are composed of two or more tightly linked genes, which can be separated by recombination. Besides, four of the thirteen variants were identified as resistant to a rust biotype for which neither of the parents is resistant. Analysis of the flanking markers of these four novel genes indicated that they arose by crossing-over. It has also been suggested that disease resistance gene may even evolve to recognize a different pathogen species. However, there is limited evidence supporting this hypothesis. Identifying, cloning and sequencing of linked genes conferring resistance to different pathogens may demonstrate such a common evolutionary origin.

A classical example is the nematode resistance gene, *Mi*-1, in tomato which possess dual resistance specificities. Cloning of *Mi*-1 gene showed that this gene belongs to the leucine zipper/nucleotide binding site/leucine –rich-repeat class of resistance genes (Milligan *et al*. 1998). At least 7 homologues have been identified for *Mi*-1, spanning a 650 kb introgressed region from *Lycopersicon peruvianum*. Two genes *viz., Mi*-1.1 and *Mi*-1.2 have been identified at the *Mi*-1 locus. The nematode resistance is provided by *Mi*-1.2 whereas the *Meu*, tightly linked to *Mi-1* locus provides resistance to the potato aphid, *Macrosiphum euphorbiae* (Thomas). Thus, resistance to the potato aphid is always associated with the presence of the nematode resistance gene, *Mi*. It has also been reported that the *Mi*-1.2 also provides an isolate-specific resistance to the potato aphid. The dual specificity of the *Mi*-1 may be attributed to similar mechanisms of feeding by both nematode and aphids (Rossi *et al*. 1998).

Illegitimate recombination (IR) is also a major evolutionary mechanism for initiating size variation in plant resistance genes. Wicker *et al*. (2007) reported IR as major mechanism that generates duplications within the LRR domain leading to the molecular diversity in the evolution of resistance gene analogues.

6. Diversification of Resistance Gene by Transposable Elements

In plants, transposable elements play a major role in the reconstruction of the genomes in response to environmental stresses such as tissue culture, irradiation or pathogen infection (Ronald 1998). Broad-spectrum elicitors of microbial origins induce the transcription of the tobacco retro transposon *Tnt1* (Pouteau *et al*. 1994). The insertion and excision of transposable elements from the regulatory and coding regions can also change the expression patterns of the gene. In maize fungal resistance gene *Hm1*, conferring resistance to *Cochliobolus carbonum* race 1, a 315 bp insertion (dHBr) was found in a mutant allele of this gene (Johal and Briggs 1992). The insertion of a transposable element (a 256 bp element named *Drone*) that disrupted the *Hm1* in an inbred line of maize lead to the susceptibility of this line to leaf spot and ear rot disease (Multani *et al*. 1998).
In rice, transposable elements appear to be the major source of variability of the *Xa21*-gene family members. A total of 17 transposable elements sequences have been grouped into 11 families. This includes three families of miniature inverted repeat transposable elements (MITEs), five novel elements, Ds-like elements, a CACTA-like element and a retro transposable element are present at the *Xa*21 locus (Song *et al*. 1998). Integration of two of these elements into coding sequences creates open reading frames that encoded truncated proteins. The insertion of the transposable element, *Retrofit* into the member of *Xa*21D generated a truncated protein lacking membrane spanning domain. The truncated ORF from this family member can confer an attenuated resistance with *Xa*21 specificity. Another transposable element insertion was reported in the member E of *Xa*21 family. This created a truncated protein that now resembles one of the *Cf* classes of resistance

genes. No resistance phenotype was however associated with member E of *Xa21* gene.

Transposable elements (TEs) can drive evolution by creating epigenetic variation. A unique mechanism for regulation of plant immune surveillance gene expression using adaptive TE insertions carrying epigenetic information was recently demonstrated in *Arabidopsis thaliana* disease resistance gene*RPP*. It has also been shown that, the function of *RPP7* is dependent on high levels of H3K9me2 at *COPIA-R7* because of epigenetic regulation (Tsuchiya and Eulgem 2013).

7. Population Dynamics of a Rice Blast Resistance Gene *Pi54*

Population dynamics of disease resistance gene is an important aspect of evolutionary studies. Two principal strategies employed for such studies are allele mining and eco-tilling. It helps to gain knowledge on the structural differences, relatedness and evolutionary aspects of the genes. *Pi54* (formerly *Pi-kh*) confers broad spectrum resistance to different strains of *Magnaporthe oryzae* in India (Sharma *et al*. 2005; Sharma *et al*. 2010; Rai *et al*. 2011). This gene has been shown to regulate the regulators of defence response genes in rice (Gupta *et al*. 2012a). It has a unique and smallest zinc finger domain among all the genes cloned till date (Gupta *et al*. 2012b). Since the gene is functionally validated and well characterized, its sequence information can be effectively used for allele mining. Allele mining for blast resistance gene *Pi54* from selected set of genotypes comprising of cultivated rice lines and wild rice species could relate structural variation of the allele to the phenotypes. There was high sequence variation between cultivated and wild species (35-90 %) than among cultivated species (1-20 %) only (Rai *et al*. 2013). The *Pi54* alleles have 142 polymorphic sites with average nucleotide diversity of 0.04522. Structural analysis of alleles showed presence of variable number of Open Reading Frames (0-2) principally having point mutations in the leucine rich repeats (LRR) regions. The Ka/Ks ratio of LRR region was >1, which shows the effect of selection pressure at this domain. The Ka/Ks ratio of coding region ranged from 0 to >1 and Tajima's D test showed negative as well as Darwinian selection within the alleles, which corresponded well with their phenotypic reaction to *M. oryzae* (Rai *et al*. 2013). Large number of SNPs and indels have also been reported in this gene which might be responsible for its effectiveness against a wide spectrum of *M. oryzae* strains prevailing in India.

8. Conclusions

Several evolutionary patterns can be inferred from the molecular studies of resistance genes. The first is the duplication events that play a significant role in the creation of resistance gene families. The duplication of progenitor resistance genes and subsequent divergence can create/amplify clusters of the genes. Secondly, unequal recombination, gene conversion at intergenic regions and illegitimate recombination create additional variability within the gene families. These events can re-assort existing resistance genes in the array of new combinations. Thirdly, intragenic recombination and gene conversion provides a mechanism for generating novel resistance specificities. Diversity at the LRR domains provides an evolutionary advantage for recognizing, binding and defending against a broad array of pathogens. Finally, the mobility of transposable elements in the genome may result in further allelic diversity, either by disrupting genes, or by influencing recombination or other chromosomal rearrangements such as translocations. Many of the processes involved in the R-gene evolution and their molecular basis are now beginning to be unraveled. Recently, a web-based plant resistance gene database, PRGdb (http://prgdb.org) has been developed which provides a comprehensive overview of the resistance genes (Sanseverino *et al*. 2013). Plant resistance gene database (PRGdb 2.0) currently have information of 112 known and 105692 putative *R*-genes present in 237 plant species which confers resistance to 124 different pathogens.

In the last few decades, disease resistance breeding has been limited to the use of sexually compatible wild species that can recognize and resist infection, without prior knowledge of corresponding *R*-Avr genes. The application of this strategy is slow

and field efficacy of *R* genes is reduced as pathogens can mutate to overcome them. Deployment of multiple, stacked *R* genes that recognize core effectors shall help reduce the chance of resistance breakdown. Alternatively, identification and editing within disease- susceptibility genes may help to limit the pathogen virulence and thereby lessening the damage to plants.

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