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Genetics of plant–pathogen interactions and resistance

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Abstract

Despite substantial advances in plant disease control strategies, our global food supply is still threatened by a multitude of pathogens and pests. Plant diseases can dramatically reduce crop yield and the impact of disease outbreaks is particularly acute in developing nations. The growing human population will require a significant increase in agricultural production. This challenge is made more difficult by the fact that changes in the climatic and environmental conditions under which crops are grown have resulted in the appearance of new diseases, whereas genetic changes within the pathogen have resulted in the loss of previously effective sources of resistance. To help meet this challenge, advanced genetic and statistical methods of analysis have been used to identify new resistance genes through global screens, and studies of plant–pathogen interactions have been undertaken to uncover the mechanisms by which disease resistance is achieved. The deployment of major, race-specific and partial, race-nonspecific resistance, either by conventional breeding or transgenic approaches, will enable the production of crop varieties with effective resistance without impacting on other agronomically important crop traits. Plant diseases can drastically abate the crop yields as the degree of disease outbreak is getting severe around the world. With the onset of recent genomic, bioinformatics and molecular biology techniques, it is quite possible to tame the R-genes for efficiently controlling the plant diseases caused by pathogens. This review summarizes the genetics of plant pathogen interaction and resistance.

Keywords: Genomics, Plant pathogen interaction, Resistance, R-genes.

Introduction

Plant pathogen interaction is a well understood mechanism which involves the activation of signals sometimes resulting in a rapid defense response against an array of plant pathogens. This response helps the host plant to avoid further infection of the disease. Induction of plant defense signaling involves the recognition of specific pathogen effectors by the products of specialized host genes called R-genes (Belkhadir *et al.,* 2004). Numerous individual plant resistance (R) genes have already been characterized and are being efficiently used in crop improvement research programs. Using plant resistance genes for developing disease-resistant varieties is a convenient alternative to other measures like pesticides or other chemical control methods employed to protect crops from diseases.

Benefits of using the plant resistance genes in resistance breeding programs include the efficient reduction of pathogen growth, minimal damage to the host plant, zero input of pesticides from the farmers and most importantly the environment friendly nature of such crops. However, in case of conventional breeding for resistance, the introgression of resistance genes from one species into the gene pool of another by repeated backcrossing is a long-term process which usually takes many hybrid generations before the backcrossing occurs. It is assumed that the complete functional studies, cloning, characterization and genetic transformation of plant resistance genes could help the researchers to overcome these problems in near future (Jonse and Dangel, 2006).

Efficient and sustained control of pathogens such as bacteria, fungi, oomycetes, viruses and nematodes is an exigency for all agricultural systems. In spite of the continued release of new resistant cultivars, the global yield losses caused by pathogens are substantial (Baker *et al.,* 2010). Plant pathogens not only decrease the crop yields, they also lower the crop quality by releasing toxins that affect human health. Moreover, pathogens

are constantly becoming resistant to existing resistance genes and pesticides.

This situation therefore demands some alternate methods of disease control. Crop improvement programs based on plant disease resistance genes are being optimized by incorporating molecular marker techniques and biotechnology. Therefore, plant resistance genes need to be studied extensively to alleviate the existing problem of pest and diseases apart from the abiotic challenges (Leach *et al.,* 2002).

Facing selective pressure imposed by the pathogens, plants have evolved post invasion resistance mechanisms, often controlled by dominant resistance genes, whose products directly or indirectly detect specific pathogen effectors and trigger effective defense responses (Chisholm *et al.,* 2006; Jonse and Dangel, 2006). R protein-triggered resistance to various pathogens is normally race-specific and only effective against pathogen strains expressing the cognate effector protein (Avr protein) recognized by the R protein. This resistance is often associated with a hypersensitive response (HR), which is manifested as rapid death of the invaded cell and in some cases a few surrounding cells (Chisholm *et al.,* 2006; Baker *et al.,* 2010).

The structural and functional analysis of plant resistance genes and R-gene loci is relevant for assembling various resistance sources effectively and for engineering new strategies for disease resistance in agriculture. Apart from that, it is highly desirable to understand the plant pathogen interaction in order to achieve the said goals. These aspects have been discussed in detail in the present review which would be beneficial for researchers engaged in plant disease control based projects. The present review also highlights the concernment of many recent investigations regarding the plant resistance genes and their dispensation in the field of plant disease management strategies.

Plant disease resistance: Plants possess two major types of disease resistance, basal defense and R-gene mediated defense (Dangel, 2001). Basal defense, which can be a constituent of both non-host and host resistance, provides first line of defense to the infection by a wide range of pathogens. Often, the plant disease resistance is cultivar or accession specific which is referred as host resistance whereas non-host resistance is the resistance against pathogens throughout all members of a plant species (Chisholm *et al.,* 2006) that is expressed when a plant comes into contact with a pathogen which is incapable of provoking any disease (Wani, 2010). Elicitors of basal defense can be plant cell wall derived components released by hydrolytic activity of enzymes secreted by invading pathogens, but also common features of the pathogen, referred as pathogen-associated molecular patterns (PAMPs), such as lipo polysaccharides, chitins, glucans and flagellins (Morel and Dangel, 1997). Non-pathogens as well as pathogens can trigger a basal resistance in plants due to the widespread presence of these molecular components in their cells (Chisholm et al., 2006). However, adapted microbes express a suite of effector proteins that often act to suppress these defenses. Subsequently, plants have evolved other receptors (R proteins) that detect these pathogen effectors and activate strong defenses (Takahashi *et al.,* 2003).

R-gene mediated pathogen resistance: Phyto-pathogens produce certain molecules called 'effectors', encoded by Avr (avirulence) genes, which are delivered directly into the plant cells during initial stage of infection. These effectors either change the physiological state of host plant in order to benefit pathogen colonization or are used to interrupt the activation of host plant defenses (Yang *et al.,* 1997; Yang *et al.,* 2009). However, plants have subsequently developed a form of immunity that is based on perception of these proteins (Yencho, 2010) by host resistance proteins called R-gene mediated pathogen resistance.

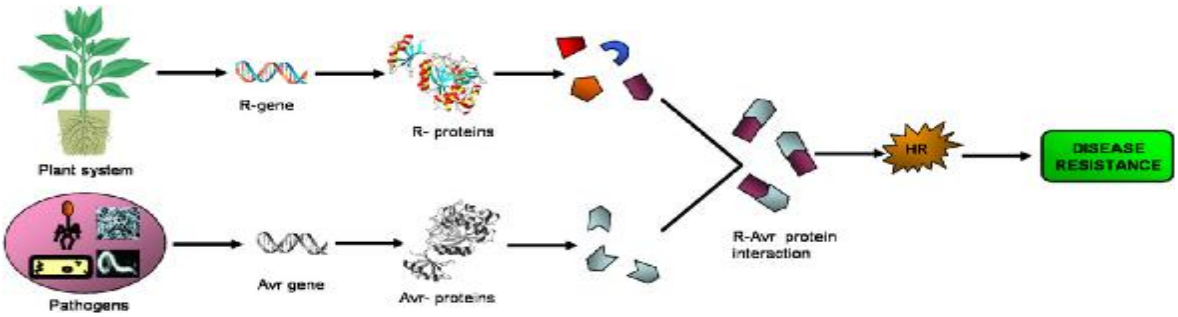


Figure (1): R-gene and Avr gene for plant disease resistance

In gene-for-gene relationships, a plant carrying a resistance gene resists pathogen races with the corresponding effectors (Yoshi *et al.,* 1998; Yoshimura *et al.,* 2004). The effectors found in bacteria, virus, nematodes, fungus, and oomycetes cause a plant pathogen to elicit a resistance response in a host plant (Figure 1). The effector genes are defined by corresponding resistance genes of which a relatively large number have now been cloned (Zahi *et al.,* 2008). This resistance response is appended with another reaction called hypersensitive reaction (HR) which is a form of programmed cell death.

The signaling cascade behind the HR is triggered either when an appropriate disease resistance gene recognizes an effector or by an elicitor of plant defense responses recognized by a specific receptor (Zhao *et al.,* 2010). Either of these signals accompanied by other factors like influx of Ca2þ ions from the extracellular space and/or anion flux results in an oxidative burst producing reactive oxygen intermediates (ROIs) and defense gene activation, finally resulting in development of local and systemic disease resistance (Baker *et al.,* 2010).

A well characterized example of HR elicitation through gene-for gene interaction is provided by the tomato (*Solanum lycopersicon*) Cf-9 gene, which confers resistance to races of the fungus *Cladosporium fulvum* expressing the Avr9 gene (Zhou *et al.,* 2010). Treatment of leaves of Cf-9 tomato or transgenic Cf-9 tobacco (*Nicotiana tabacum*) with the Avr9 peptide induces HR (Zepfel, 2008) and Avr9-treated Cf-9 tobacco cell cultures showed rapid production of ROS and activation of MAP (Mitogen Activated Protein) kinases and calcium-dependent protein kinases (Walton, 1996; Wang *et al.,* 2009). The interaction between rice (*Oryza sativa*) and the fungal pathogen *Magnoporthe grisea* (Hebert) Barr (anamorph *Pyricularia grisea* Sacc.) causing the devastating rice blast disease is another example of well documented gene-for-gene system (Wani *et al.,* 2010). *M. grisea* has the Avr-Pita gene containing the C-terminal 176 amino acids which functions as an elicitor molecule that directly binds the Pita protein of rice and triggers a signal cascade leading to resistance (Wang *et al.,* 2009).

Despite several studies and intense efforts with numerous sets of R and Avr proteins (Warren *et al.,* 1997; Weng *et al.,* 2009), the interaction between R and Avr proteins remained inexplicit and the insufficiency of verifiable R -Avr interactions led to the formulation of the guard hypothesis (Wahalen *et al.,* 1997). According to this model, the R proteins activate resistance when they interact with another plant protein known as guardee protein that is targeted and modified by the pathogen in order to create an appropriate environment. Resistance is initiated when the R protein detects an attack of its guardee or, in some cases when the R protein recognizes the product of the pathogen attack (Baker *et al.,* 2010), which might not necessarily involve direct interaction between the R and Avr proteins (Weng *et al.,* 2010).

To date, the most convincing evidence for the guard hypothesis has been found in *Arabidopsis thaliana* bacterial R-Avr systems (Baker *et al.,* 2010) where RIN4 (RPM1- interacting protein 4) was identified as a cellular protein that is required for the resistance to *Pseudomonas syringae* pv. *tomato* mediated by RPM1 and RPS2. The RIN4 (guardee) is modified in various ways, depending on the Avr that it associates with, and these modifications then serve to activate the corresponding R protein (guard). Another example is the cleavage of the *A. thaliana kinase* PBS1 (guardee) by the cysteine protease AvrPphB from *P. syringae pv. tomato*, which results in activation of RPS5 (guard)- mediated resistance (Warren *et al.,* 1997). Recently, it was shown that AvrPphB, a cysteine protease, binds PBS1 and cleaves it, which triggers RPS5- mediated resistance, indicating that RPS5 might sense the integrity of PBS1 (Whitham *et al.,* 1997; Acciarri *et al.,* 2007).

Several genes have been implicated in the regulation of resistance gene function; of these, Rar1 and Sgt1 are among the most extensively studied genes. It has been reported that Rar1 and Sgt1 are required in multiple R-gene mediated and non-host resistance responses to a variety of pathogens (Albar *et al.,* 2006). A notable example is in barley where the regulation of Mla transcript accumulation is not constitutive and that induction is coordinately controlled by recognition-specific factors (Alfano and Colmer, 1994). Rar1 from barley has been identified as a required component for resistance against powdery mildew (*Blumeria graminis f. sp. Hordei)* mediated by Mla12 (Allen *et al.,* 2004) which is required for a subset of R-gene mediated resistance responses in monocot and dicot plant species (Armstrong *et al.,* 2005). Sgt1 interacts with Rar1, and contributes to R gene mediated resistance (Zhao *et al.,* 2010) although recently, Bhaskar *et al.* (2010) demonstrated that Sgt1, but not Rar1, is essential for the RB-mediated broad-spectrum resistance to potato late blight. Similarly, Austine *et al.* (2003) reported that Hsp90 (heat shock protein 90), a molecular chaperone and one of the most abundant proteins expressed in cells was found as

a required component for Mla13- mediated race-specific resistance.

Major classes of R genes: Plant resistance genes can be broadly divided into eight groups based on their amino acid motif organization and their membrane spanning domains. The LRRs (Leucine rich repeats) represents the components having an important role for recognition specificity and these domains are present in the majority of R proteins (Baker *et al.,* 2010). First major class of R-genes include the genes encoding for cytoplasm proteins with a nucleotide-binding site (NBS), a C-terminal leucine rich repeat (LRR) and a putative coiled coil domain (CC) at the N- terminus. The examples of this class of resistance genes include the *P. syringae* RPS2 and RPM1 resistance genes of Arabidopsis and the tomato *Fusarium oxysporum* resistance gene I2. The second class of resistance genes consists of cytoplasmic proteins which possess LRR and NBS motifs 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 a few examples categorized under this class (Wang *et al.,* 2010; Zhao *et al.,* 2010).

Third major class of resistance genes family devoid of NBS motif consists of extra cytoplasmic leucine rich repeats (eLRR), attached to a transmembrane domain (TrD). eLRRs are known to play an important role for certain defense proteins such as, polygalacturonase inhibiting proteins (PGIPs) (Weng *et al.,* 2006) even though they are not directly involved in pathogen recognition and activation of defense genes (Austin *et al.,* 2003). The C. fulvum resistance genes (Cf-9, Cf-4 and Cf-2) having an extracellular LRR (eLRR), a membrane spanning domain, and a short cytoplasmic C terminus (Armstrong *et al.,* 2005) are some examples of this class of resistance genes. The rice Xa21 resistance gene for Xanthomonas is an example of the fourth class of resistance genes which consists of an extracellular LRR domain, a transmembrane domain (TrD) and an intracellular serine-threonine kinase (KIN) domain (Morel and Dangel, 2005).

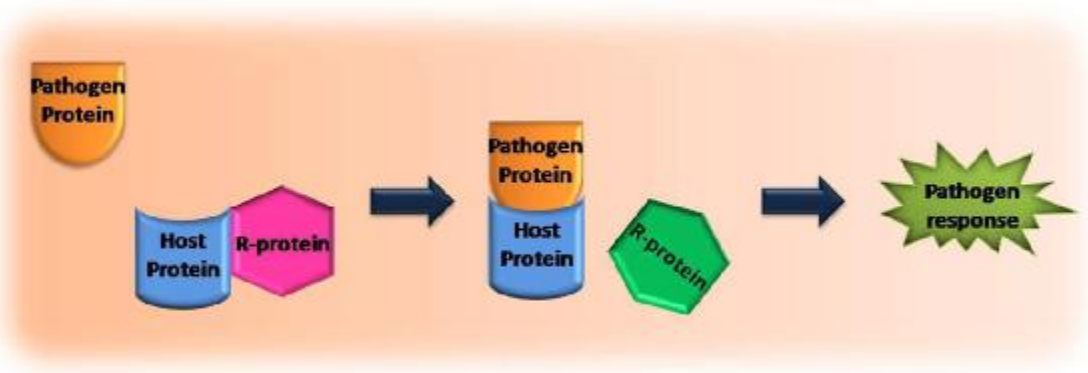


Figure (2): Host pathogen protein interaction

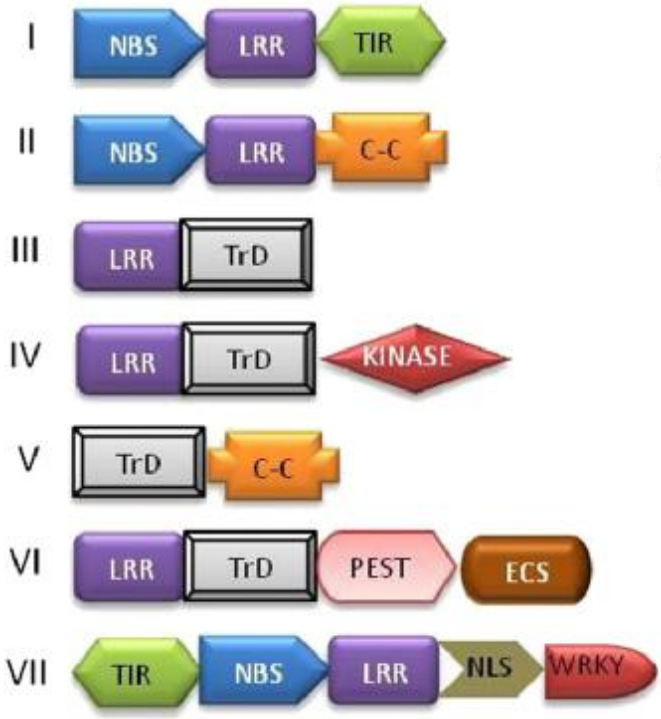
The fifth class of resistance genes contain the putative extracellular LRRs, along with a PEST (Pro-Glu-Ser-Thr) domain for protein degradation (found only in Ve2, and not Ve1), and short proteins motifs (ECS) that might target the protein for receptor mediated endocytos (e.g. tomato Ve1 and Ve2 genes) However, these Ve1 and Ve2 proteins have recently been proposed as PAMP receptors (Armstrong *et al.,* 2005).

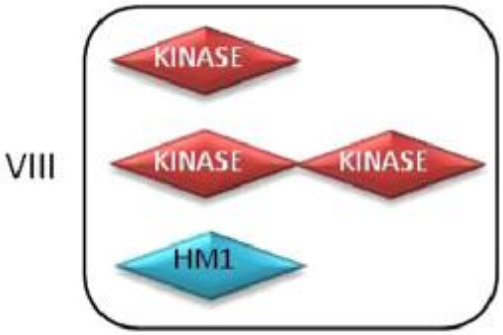
The Arabidopsis RPW8 protein is an example of the sixth major class of resistance genes which contains a membrane protein domain (TrD), fused to a putative coiled coil domain (CC) (Albar *et al.,* 2006) whereas, the seventh major class of resistance genes includes the Arabidopsis RRS1-R gene conferring resistance to the bacterial phytopathogen *Ralstonia solanacearum*, and it is a new member of the TIReNBSeLRR R protein class. RRS1-R has a C-terminal extension with a putative nuclear localization signal (NLS) and a WRKY domain (Zhao *et al.,* 2010). The WRKY domain is a 60 amino acid region that is defined by the conserved amino acid sequence WRKYGQK at its Nterminal end, together with a novel zinc-finger-like motif.

The eighth major class of resistance genes includes the enzymatic R-genes which contain neither LRR nor NBS groups. For example the maize Hm1 gene which provides protection against southern corn leaf blight caused by the fungal pathogen *Cochliobolus carbonum* (Zhao *et al.,* 2010). Unlike other resistance genes, Hm1 encodes

the enzyme HC toxin reductase, which detoxifies a specific cyclic tetrapeptide toxin produced by the fungus (HC toxin) that is essential for pathogenicity.

Cereal resistance genes like Hm1 can be seen to encode a range of different proteins that in some cases have obviously very different functions. Another notable example, Pto protein in *P. syringae* contains a Ser-Thr kinase domain without LRRs (Zhao *et al.,* 2010) whereas, the Rpg1 gene of barley which confers resistance to stem rust encodes a receptor kinase-like protein with two tandem protein kinase domains and does not contain a strong membrane-targeting motif and known receptor sequences (Zhao *et al.,* 2010).





Though most of the resistance genes show dominant inheritance, recessive resistance is fairly common in viral systems. Recessive resistance genes in bacterial and fungal plant pathogen interactions have also been reported, such as barley mlo, Arabidopsis RRS1-R, rice xa13, and xa5 (Warren *et al.,* 1997; Weng *et al.,* 2009). With the onset of functional genomics approaches and complete genome sequencing of some important crop plants, the identification and deployment of R-genes has become easier. Numerous resistance genes conferring resistance against a range of pathogens have been successfully used in development of transgenic crops. Therefore, the possibility of discerning some novel classes of resistance genes in near future cannot be ruled out.

Genes for bacterial disease resistance: A number of plant resistance genes conferring resistance against bacterial attack have been studied so far for the majority of plant diseases, the genetics of susceptibility are less tangible. It has been known that bacterial pathogens of both plants and animals deliver virulence proteins into the host cytoplasm via the type-III secretion system (T3SS), also called injectisome (Zhao *et al.,* 2010) which enables Gram negative bacteria to secrete and inject pathogenicity proteins into the cytosol of eukaryotic host cells (Wang *et al.,* 2010; Zhao *et al.,* 2010)

The T3SS is encoded by hrp (HR and pathogenicity) and hrc (HR and conserved) genes, whose mutations eliminate bacterial pathogenicity in susceptible host plants and the ability to elicit HR in non-host or cultivar-specific resistant plants. Many of the T3SS effector proteins have been shown to be dependent on molecular chaperones, which keep the effector in a partially unfolded form in the bacterial cytoplasm (Wang *et al.,* 2010; Zhao *et al.,* 2010). The emergent results on their role in pathogenesis have indicated that they act as molecular double agents betraying the pathogen to plant defenses in some interactions and suppressing host defenses in others.

In rice, resistance and susceptible alleles of Xa27 encode identical proteins however; expression of

only the resistance allele occurs when a rice plant is challenged by bacteria harboring AvrXa27, whose product is a nuclear localized T3SS effector. Induction of Xa27 occurs only in the immediate vicinity of infected tissue, whereas ectopic expression of Xa27 results in resistance to otherwise compatible strains of the pathogen. The Xa27 specificity toward incompatible pathogens involves the differential expression of the resistance gene in presence of the AvrXa27 effector (Zhao *et al.,* 2010). A dominant rice gene Os8N3 is an exception as it is up- regulated by a bacterial type-III effector protein, and that confers gene-for-gene specified disease susceptibility (Whitham *et al.,* 1997 Acciarri *et al.,* 2007).

Some bacterial resistant plant resistance genes may confer resistance against unrelated or distantly related pathogens. Zhao *et al.* (2003) demonstrated the feasibility of non-host resistance gene transfer between two cereal crops maize and rice. They proposed that a maize non-host resistance gene Rxo1 recognizes a rice pathogen, *Xanthomonas oryzae* pv. *oryzicola* and causes bacterial streak disease. Interestingly, Rxo1 was also found to confer resistance to the unrelated pathogen *Burkholderia andropogonis*, known to cause bacterial stripe of sorghum and maize indicating that the same gene controls resistance to both pathogens and non-pathogens of maize. The function of Rxo1 in rice thus demonstrates that an NBS-LRR type of resistance gene can be effectively transferred between distantly related cereals (Whitham *et al.,* 1997 Acciarri *et al.,* 2007).
Genes for fungal disease resistance: Fungal diseases are rated either the most important or second most important factor contributing to yield losses in almost all the major crops (Baker *et al.,* 2010). So far, several fungal resistance genes have been reported and used in crop improvement programs.

However, the sequence variation occurring within the central LRR domain and the variation in LRR copy number of the gene plays an important role in determining recognition specificity (Wang *et al.,* 2010; Zhao *et al.,* 2010). For example, the sequence variations in tomato Cf-4 and Cf-9 genes play an important role in determining recognition specificity, which confer resistance to biotrophic leaf mold pathogen Cladosporium and induce a hypersensitive response (HR) upon recognition of the fungus-encoded Avr4 and Avr9 peptides.

In tomato, Ve is involved in race-specific resistance to infection by Verticillium species (Armstrong *et al.,* 2005). The Ve1-mediated resistance signaling only partially overlaps with signaling mediated by Cf- proteins (Wang *et al.,* 2010). Recently, a virus induced gene silencing approach for the characterization of Ve mediated signaling revealed that signaling cascade downstream of Ve1 requires two genes EDS1 (Enhanced Disease Susceptibility 1) and NDR1 (non-race-specific disease resistance 1).

Moreover, the results showed that the locus Ve consists of two closely linked inversely oriented genes, Ve1 and Ve2 encoding cell surface receptor proteins of the extracellular LRR receptor-like protein. Out of them, only Ve1 provides resistance in tomato against race 1 strains of *Verticillium dahliae* and *Verticillium albo-atrum* and not against race 2 strain. Based on the sequence analysis and the expression study, Ve1 and Ve2 expression is induced in resistant as well as susceptible tomato genotypes and that no single mutation in the CDS of Ve2 discriminates resistant and susceptible tomato genotypes. However, a single point mutation in Ve1, resulting in a premature stop codon, was found in all susceptible genotypes and was absent in all resistant genotypes. This suggested that Ve1, but not Ve2, governs Verticillium resistance in tomato (Wang *et al.,* 2010; Zhao *et al.,* 2010).

A disease epidemic broke out in oats in the 1940's due to the extensive planting of ''Victoria-type'' oats carrying the Pc-2 gene for resistance against the rust fungus, *Puccinia coronata*. Oats carrying Pc-2 were highly susceptible to another disease, Victoria blight, caused by a fungus *Cochliobolus victoriae* (Wang *et al.,* 2010; Zhao *et al.,* 2010). Pathogenicity of *C. victoriae* is dependent on the production of a toxin called victorin, and in oats, both toxin sensitivity and Victoria blight disease susceptibility are conferred by the dominant Vb gene. Despite extensive efforts, rust resistance (Pc-2) and Victoria blight susceptibility (Vb) have not been genetically separated and are suspected to share identity thus suggesting an unexpected relationship between plant disease resistance and susceptibility (Weni, 2010).

Stem rust-susceptible barley cv. Golden Promise was transformed into a highly resistant one to pathotype Pgt-MCC of the stem rust fungus *Puccinia graminis* f. sp. tritici by Agrobacterium mediated transformation with the dominant Rpg1 gene. A single copy of Rpg1 against stem rust, and progenies from several transformants segregated in a 3:1 ratio for resistance: susceptibility as expected for Mendelian inheritance and unequivocally demonstrated that the DNA segment isolated by map-based cloning is the functional Rpg1 gene for resistance to stem rust and the transformants exhibited a higher level of

resistance than the original sources of Rpg1 like cvs. Chevron and Peatland (Warren *et al.,* 1997; Weng *et al.,* 2009). Another fungal resistance plant resistance gene RUS1 from *Setaria italica* Beauv. cv. Shilixiang resistant to Uromyces *S. italica*, was cloned and it was found to contain an NB- ARC (nucleotide-binding adapter shared by APAF-1, R proteins, and CED-4) domain as well as three conserved motifs P-loop, kinase 2, and kinase 3, having the characteristics of NBS-LRR type resistance gene of plant (Morel and Dangel, 2005).

Another notable example of fungal resistance genes is the broad-spectrum mildew resistance gene RPW8.2 from Arabidopsis thaliana which is induced by powdery mildew and is assumed to be involved in enhancing the formation of a callosic encasement of the haustorial complex (EHC) with onsite accumulation of H_2O_2, in order to constrain the haustorium while reducing oxidative damage to the host cell. Targeting of RPW8.2 to the EHM (Extra haustorial membrane) requires normal function of the actin cytoskeleton while microtubules are not involved in the process (Weng *et al.,* 2009).

Despite its critical role for the defense function, SA signaling is dispensable for targeting RPW8.2 to the EHM and both EHM localization and defense activation are required for RPW8.2 to induce resistance against powdery mildew (Wnag *et al.,* 2010). The majority of resistance genes reside in clusters, and the frequency of recombination between clustered genes can vary remarkably, even within a single cluster. The Apple Vf locus, derived from the crab apple species *Mauls floribunda*, confers resistance to five races of the apple scab fungus *Venturia inaequalis*. The Vf locus comprises a cluster of four RLP genes, HcrVfa1 to HcrVfa4 (forhomolog of the *C. fulvum* resistance genes of the Vf region), of which HcrVfa1, HcrVfa2 and HcrVfa4 encode typical RLPs while HcrVfa3 contains an insertion at the end of the LRR motif, resulting in truncated transcripts (Zhao *et al.,* 2010). Only expression of HcrVfa1 or HcrVfa2 in susceptible apple cultivars provided resistance against V. inaequalis strains (Weng *et al.,* 2005).

Genes for Oomycetes disease resistance: Phytopathogenic oomycetes are responsible for economically important diseases, such as late blight of potato and sudden oak death caused by *Phytophthora infestans* and *Phytophthora ramorum* respectively. The oomycetes (Pseudofungi) have been classified within the phylum Heterokontophyta comprising a number of microbial lineages with phenotypic similarities to true fungi. It was only with the use of molecular phylogenetic methods starting with small subunit rDNA analysis followed by multiple concatenated gene phylogenies that the oomycetes were demonstrated to group within the heterokont radiation. Several functional resistance genes from potato conferring resistance to late blight have been cloned and all of them belong to the NBS-LRR class of plant resistance genes (Wang *et al.,* 2010; Zhao *et al.,* 2010).

In addition to the resistance to *P. infestans* genes Rpi-blb1 (RB) and Rpi-blb2, *Solanum bulbocastanum* appears to harbor Rpi-blb3 located at a major late blight resistance locus on LG IV, which also harbors Rpi-abpt, R2, R2- like, and Rpi-mcd1 in other Solanum sp. Vleeshouwers *et al.* (2005) used a candidate gene approach for the rapid cloning of *S. stoloniferum* Rpi-sto1 and *S. papita* Rpi-pta1, which are functionally equivalent to Rpi-blb1. Cloning and functional analyses of four Rpi genes, Rpi-blb3, Rpi-abpt, R2, and R2-like revealed that these genes contain all signature sequences characteristic of leucine zipper nucleotide-binding site leucine rich repeat (LZNBS-LRR) proteins, and share 34.9% of amino acid sequences similar to RPP13 from *A. thaliana* (Wang *et al.,* 2010; Zhao *et al.,* 2010).

So far, a number of *Hyaloperonospora parasitica* resistance (RPP) genes against the downy mildew have been cloned from Arabidopsis which belong to the NBS-LRR class of resistance genes. These resistance genes are distinguished by their N-terminal regions, showing homology to the TIR domain (RPP1 and RPP5 clusters) and leucine zipper motifs (RPP8 cluster. Another example of oomycetes resistance genes with NBS-LRR motifs is downy mildew resistance gene, Dm3 (Wang *et al.,* 2010; Zhao *et al.,* 2010) in *Bremia lactucae* which is a member of the large RGC2 (Resistance Gene Candidate2) multigene family similar to the genes cloned from other species for resistance to downy mildews and other pathogens. Several oomycete effector genes encoding products that are recognized by R proteins situated in the plant cytoplasm have been discovered which indicate toward a mechanism of transporting fungal and oomycete effectors into plant cells (Wang *et al.,* 2010; Zhao *et al.,* 2010).

This mechanism has recently been characterized using gene ontology by Torto-Alalibo *et al.* (2005) while the motifs in their amino acid sequence have already been identified in the past (Zepfel, 2008). The identification of the first effectors from oomycetes, together with whole genome sequencing projects has revealed a special class of secreted effector proteins, RXLR that are delivered into host cells (Zhao *et al.,* 2010). The RXLR effectors constitute large super families of rapidly

evolving proteins in all oomycete genomes (Whitham *et al.,* 1997; Acciarri *et al.,* 2007) and include Avr1b-1, Avr1a and Avr3a from Phytophthora sojae (Beker *et al.,* 2010), Avr3a, Avr4, and Avrblb1 from *P. infestans*, ATR1 and ATR13 from *Hyaloperonospora arabidopsidis* and IpiO and IpiB from certain Phytophthora species including *P. infestans* (Zhao *et al.,* 2010). While the majority of IPI-O proteins are recognized by RB gene to elicit host resistance, some variants exist that are able to elude detection (e.g. Ipi-O4). Intriguingly, few oomycete effectors that do not encode RXLR effectors have also been proposed, such as Avr3b, Avr10 and Avr11 in *P. infestans* and Avr1b-2 in *P. sojae* (Wang *et al.,* 2010).

So far, the host targets of RXLR effectors have not been well described in the literature , while the target proteins of several oomycete apoplastic effectors have been determined (Warren *et al.,* 1997; Weng *et al.,* 2009). P. sojae encodes numerous putative host cytoplasmic effectors with conserved FLAK (F, Phe; L, Leu; A, Ala and K, Lys) motifs following signal peptides, termed crinkling- and necrosis inducing proteins (CRN) or Crinkler. Recently, the functional studies of CRN revealed that two functional genes, PsCRN63 and PsCRN115 encode proteins that induce contrasting responses when expressed in *Nicotiana benthamiana* and soybean (Glycine max). Silencing of the PsCRN63 and PsCRN115 genes in *P. sojae* stable transformants exhibited a reduction of virulence on soybean and a loss of ability to suppress host cell death and callose deposition on inoculated plants. These results suggested a role for CRN effectors in the suppression of host defense responses (Warren *et al.,* 1997; Weng *et al.,* 2009). In future, more studies on oomycete effectors and their cognate host targets will undoubtedly explore novel plant immune pathways.

Genes for nematode disease resistance: Plant parasitic nematodes are obligate parasites that obtain nutrition from the cytoplasm of living plant cells and comprise many species including ectoparasites and endoparasites. Nematode resistance genes are present in several crop species and form an important component in many breeding programs including those for tomato, potato, soybeans and cereals (Whitham *et al.,* 1997; Acciarri *et al.,* 2007). Numerous sources of nematode resistance have been identified and several of the responsible genes have been genetically mapped]. Resistance to root-knot nematode was first identified in *Lycopersicum peruvianum* Mill., a wild relative of cultivated tomato .

The single dominant Mi gene of tomato confers resistance to three major root-knot nematodes *Meloidogyne arenaria, Meloidogyne incognita* and *Meloidogyne javanica* but it does not confer resistance to *Meloidogyne hapla*, a nematode present in overlapping geographic locations. Mi gene encodes a protein with CC-NBSLRR motifs (Morel and Dangel, 2005) was introduced into cultivated tomato using embryo culture of an interspecific cross between *Lycopersicum esculentum* and *L. peruvianum*, followed by extensive backcrossing with *L. esculentum*. Later this gene was isolated by positional cloning approach. Mi-1 confers resistance to the root knot nematodes. The mechanism of resistance to nematodes conferred by Mi appeared to involve a hypersensitive response on the part of the host. Mi-1 remains the only cloned root-knot nematode resistance gene and the resistance mediated by Mi- 1 acts in a gene-for-gene manner (Warren *et al.,* 1997; Weng *et al.,* 2009).

Several common components that interact with R proteins or required for resistance gene function have been recently identified. Bhattarai *et al.* (2005) demonstrated the role of Hsp90, Sgt1, and Rar1 in Mi-1-mediated aphid and nematode resistance. Studies with approaches however identified the requirement of Rme1 gene for Mi-1-mediated resistance to nematodes, aphids, and whiteflies.

In addition to Rme1, Mi-1 resistance requires the salicylic acid (SA) signaling pathway and mitogen activated protein kinase (MAPK) cascades. The tomato MAPK kinases MKK2 and MAPKs LeMPK1, LeMPK2, andLeMPK3 are required for Mi-1- mediated aphid resistance. However, their role in root-knot nematode resistance has not yet been identified. The first nematode resistance gene to be cloned was Hs1pro-1, a gene from a wild relative of sugar beet conferring resistance against *Heterodera schachtii*, the beet cyst nematode. Hs1pro-1 cloned under the control of the CaMV35S promoter, was shown to confer nematode resistance to susceptible sugar beet roots transformed with *Agrobacterium rhizogenes* however, the resistance mediated by Hs1pro-1, does not appear to involve a hypersensitive response (Acciarri *et al.,* 2007).

Complementation analysis by stable potato transformation showed that the gene Gro1-4 conferred resistance to *Globodera rostochiensis* pathotype Ro1 and it encodes a protein of 1136 amino acids containing the TIR, NBS and LRR homology domains along with a C-terminal domain with unknown function (Baker *et al.,* 2010). The Gpa2 gene that confers resistance against some isolates of the potato cyst nematode *Globodera*

pallida, is a member of the NBS-LRR-gene family and contains a possible LZ near its amino terminus. Gpa2 is highly similar in predicted amino acid sequence to the Rx1 gene which confers extreme resistance to Potato Virus X (Warren *et al.,* 1997).

The Cre3 gene confers a high level of resistance to the root endoparasitic nematode *Heterodera avenae* in wheat. As a result of map-based cloning of a disease resistance gene family at the Cre3 locus, two genes related to members of the cytoplasmic NBS-LRR class of plant disease resistance genes have been analyzed. One encodes a polypeptide with a nucleotide-binding site (NBS) and a leucine rich region; this member of the disease resistance gene family is expressed in roots. The second Cre3 gene sequence appears to be a pseudo gene, with a frame shift caused by a deletion event (Jones and Dangel, 2001). Based on the conserved regions of known resistance genes, an NBSeLRR-type CCN (cereal cyst nematode) resistance gene analog was isolated from the CCN resistant Ee10 near isogenic lines (NILs) of wheat, designated as CreZ. The expression profiling of CreZ indicated that it was specifically expressed in the roots of resistant plants and expression levels drastically increased when the plants were inoculated with cereal cyst nematodes (Warren *et al.,* 2010).

In addition, the wheat and barley resistance gene analogs (RGAs) contain other conserved motifs present in known resistance genes from other plants and share between 55 and 99% amino acid sequence identity to the NBS-LRR sequence at the Cre3 locus and have been found to be associated with CCN and aphid resistance in barley (Zhao *et al.,* 2010). In another example, a candidate root-knot nematode resistance gene (designated as CaMi) was isolated from the resistant pepper line PR 205 which was highly expressed in roots, leaves, and flowers, and at a lower level in stems, and not detectable at all in fruits. Transgenic plants expressing CaMi gene triggered a hypersensitive response (HR) as well as many necrotic cells around nematodes and thus conferred significant resistance to root-knot nematodes when compared to susceptible control plants (Chisholm *et al.,* 2006; Baker *et al.,* 2010).

Viral resistance genes: The majority of characterized viral resistance genes from plants fall into the NBS-LRR class of resistance genes, providing monogenic dominant resistance (Table 6). Although, these R proteins appear to be similar, they confer resistance to highly divergent viruses. For example, A. thaliana RCY1 (resistance to C strain Y1) and HRT (HR to turnip crinkle virus) are allelic, encode proteins that share 91% similarity but confer resistance to unrelated viruses such as cucumber mosaic virus (CMV, a cucumovirus) and turnip crinkle virus (TCV, a carmovirus), respectively.

The viral R protein-Avr system that strongly justifies the guard hypothesis is the HRT-TCV pair. The TCV coat protein is the Avr determinant for HRT-mediated resistance responses and its interaction with a host transcription factor, TCV-interacting protein (TIP) is required for HRT-elicited defense responses (Weng *et al.,* 2010). Although, a direct interaction between HRT and TIP has not been reported, TCV coat protein inhibits the nuclear localization of TIP, however it is possible that HRT detects the altered cellular distribution of TIP which might therefore be the guardee of the guard protein HRT.

However knock out mutation studies showed that loss of TIP does not alter HR or resistance to TCV. Moreover, the mutation in TIP neither impaired the salicylic acidemediated induction of HRT expression nor the enhanced resistance conferred by over expression of HRT. Noticeably, the mutation in TIP resulted in increased replication of TCV and Cucumber mosaic virus, suggesting that TIP may play a role in basal resistance but is not required for HRT-mediated signaling.

Resistance to Tomato Spotted Wilt Virus (TSWV) in tomato is conferred by Sw-5 gene which was introgressed from *Solanum peruvianum* into tomato, and has demonstrated broad and stable resistance. The positional cloning of Sw-5 locus was revealed that the resistance allele encodes a CC-NBS-LRR R protein and is remarkably similar to the tomato Mi gene for nematode resistance with the exception of four leucine zippers at the N terminus (Yang *et al.,* 2009).

In cultivated tomato, To MV (Tomato mosaic virus) infections are controlled by the introgressed Tm-1, Tm-2 and Tm-22 genes. The Tm-22 resistance gene was shown to be strikingly durable and it has been cloned and well characterized by Lanfermeijer. The susceptible tomato plants, which were transformed with the Tm-22 gene, displayed resistance against To MV infection and the resistance was conserved in all transgenic lines.

A common strategy proposed to achieve broad-range host resistance is to modify the narrow pathogen specificity of R-gene mediated resistance. Therefore, elucidation of R protein domains that control recognition of specific pathogens and subsequent activation of the downstream defense response has been the subject of intense research. The function of a particular resistance gene totally depends on the

pathogen's genotype but there are some resistance genes which confer resistance against a broad range of pathogens.

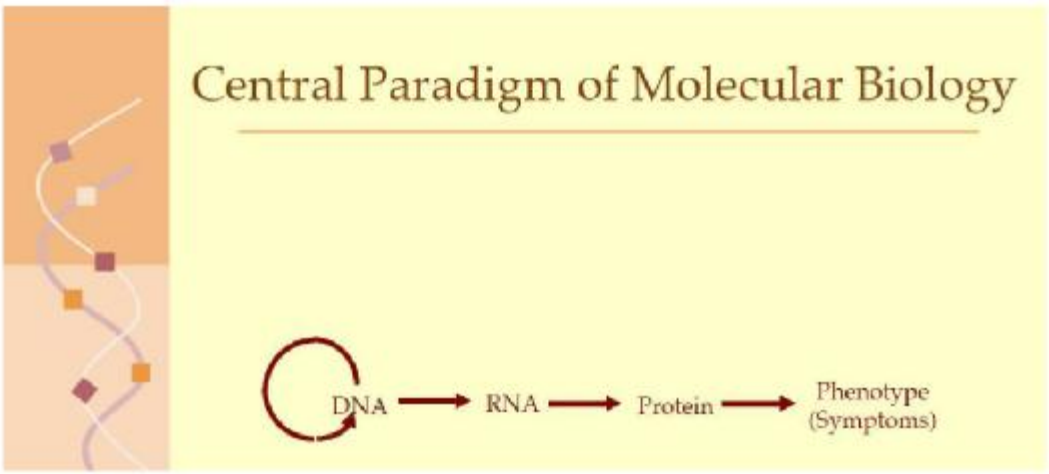
Conclusions

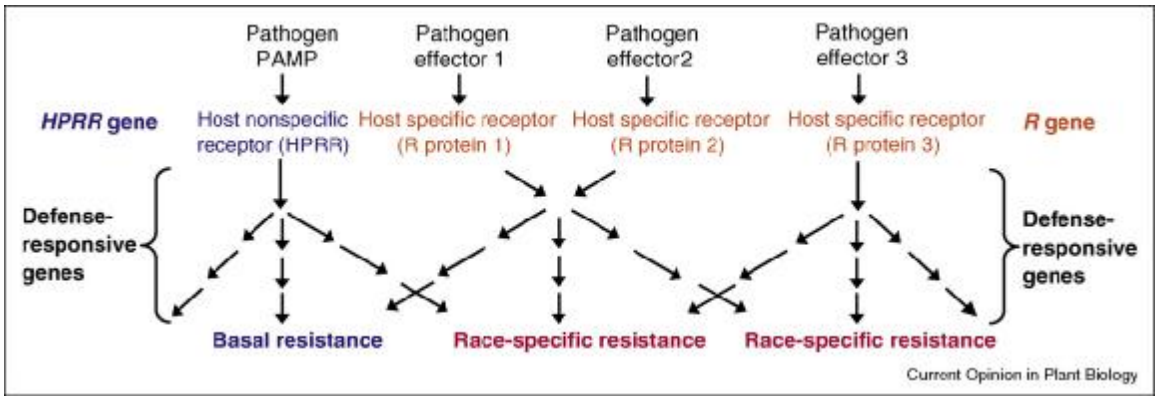
With the advent of high throughput techniques and efficient genomic approaches, researchers have managed to produce a large amount of experimental data in the form of ESTs, whole genome sequences, gene expression data etc. Still, the progress in understanding the functional mechanism of resistance genes has been moderate. For instance, little is known about the structural basis of pathogen recognition. Furthermore, there is still an inadequacy of a reference set of sequences to be used as model for resistance genes that usually cluster in genomic regions with a high number of homologs and pseudo genes. The difficulties in performing the plant pathogen interaction studies pose another obstacle (Leach *et al.,* 2002). Nevertheless, efficacious applications are being continuously developed based on our rather finite knowledge base.

For example, recently PRGdb, a web accessible open source database providing a comprehensive overview of resistance genes has been developed, which is definitely going to help filling some gaps in the models of the plant defense signal transduction network. The primary benefit of deploying resistance genes in transgenic technology is its ability to overcome the fertility restraints for the dispersal of genes originating from a different species; for example, of Bs2 resistance gene was identified originally in pepper and its resistance has been found durable in the field against isolates B. campestris (Baker *et al.,* 2010).

Another advantage of resistance genes usage in transgenic technology is that it allows introducing several different resistance gene alleles, each effective against a single pathogen species or race, into semi-elite and elite germplasm. Moreover, most resistance genes exhibit exquisite recognition specificity and to overcome this deficit, new resistance genes have been created in the laboratory through single point mutations, which are auto-activating (Warren *et al.,* 2010). Cloned resistance and effector genes can be used in combination to promote acquired resistance. The rapid activation of localized defense responses at the site of pathogen infection, often associated with an HR, is the most prevalent and effective mechanism used by plants to minimize pathogen attack.

By combining R and Avr gene expression in a single plant genotype, it is possible to engineer a 'trigger' for HR . Efficient application of functional genomics tools for disease resistance could not only help us better understand the plant defense signaling, it could reveal novel insights on the interactions between these signaling pathways and other plant processes (Weng *et al.,* 2005). Even though, the progress toward the overall plant defense mechanism studies is going on at a considerable pace, it would still be imprudent to expect a great breakthrough in impervious broad-spectrum resistance. However, it is judicious to anticipate an array of highly useful tools aided by other control measures providing adequate protection in certain contexts.





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