ABSTRACT: Efforts have been on for several years now in introgressing disease-resistant genes into elite cultivars through conventional breeding. With the rapid strides made in the development of transgenic plants as well as in the understanding of disease resistance at the molecular level, it is now possible to devise more efficient strategies for the development of disease-resistant varieties. An attempt has been made in this article to describe the advances made in the basic understanding of plant disease resistance genes (R genes) - the plant’s natural arsenal against pathogens – and discuss the application of this knowledge towards enhancing disease resistance in crops. A number of R genes have been cloned and characterized in terms of their structure and function. The models proposed for interactions of R proteins and Avr proteins have now been supported by adequate molecular evidence. Both host and non-host resistance R genes have been used to engineer disease resistance in crop plants. Although our current knowledge on molecular basis of plant-pathogen interactions is only fragmentary, given the advances being made, it is reasonable to expect that in the years to come, we would be able to devise suitable strategies that will provide effective and eco-friendly protection against plant diseases.

INTRODUCTION

Ever since man started practising agriculture, he has been continually trying to evolve plant varieties with improved yield and quality. Plant diseases have always been major constraints to successful crop production. Several strategies have been adopted by man to surmount this problem. One of the eco-friendly approaches has been the development of disease-resistant plants. Efforts have been on for several years now in introgressing disease-resistant genes into elite cultivars through conventional breeding. With the rapid strides made in the development of transgenic plants as well as in the understanding of disease resistance at the molecular level, it is now possible to devise more efficient strategies for the development of disease-resistant varieties. An attempt has been made in this article to describe the advances made in the basic understanding of plant disease resistance genes (R genes) – the plant’s natural arsenal against pathogens – and discuss the application of this knowledge towards enhancing disease resistance in crops.

STRUCTURE AND FUNCTION OF R GENES

Plants exhibit different kinds of defence responses against invading pathogens. These include genetically-programmed suicide of infected cells (HR- hypersensitive response), tissue reinforcement and production of antimicrobial compounds. These local responses
in turn can trigger longer lasting systemic responses like systemic acquired resistance (SAR). One of the most important of these defence systems is the generation of 'R' proteins which are encoded by 

**resistance genes** (R genes). Each R protein typically responds to the product of 'Avr' ('avirulence') genes expressed by the pathogen during infection. The gene-for-gene hypothesis was first postulated by Flor (1956) during his pioneering classical genetic studies of the flax-flax rust interactions. According to this, a resistance to a pathogen is only observed when the pathogen carries a specific avr gene and the plant carries a corresponding resistance R gene. Subsequently, the genetic relationship between the gene products i.e. R and Avr proteins has also been elegantly demonstrated (Flor, 1971). This type of plant defence is now described as plant innate immunity (Burch-Smith et al., 2007).

The first R gene to be cloned was a protein kinase gene from tomato which was shown to confer resistance to *Pseudomonas syringae* (Martin et al., 1993). Since then, approximately 40 R genes have been cloned (Burch-Smith et al., 2007). These genes confer resistance to several classes of pathogens, including viruses, bacteria, fungi, oomycetes, insects and even nematodes. One interesting feature is that majority of R genes encode proteins with specific conserved structural domains (Lehmann, 2002). Most of them contain a conserved nucleotide-binding site (NBS) which is crucial to the binding of ATP or GTP. The leucine-rich repeat (LRR) domain is another common domain and it is also found in animal innate immunity molecules including Toll from *Drosophila* and Toll-like receptors from mammals (Burch-Smith et al., 2007). The NBS-LRR class of proteins is further subdivided according to the N-terminal domain of these proteins. Some of these contain a Toll-interleukin 1 receptor homology region (TIR) domain, while others possess a coiled-coil (CC) domain. Based on common molecular features, R genes are divided into five classes as shown in Table 1.

**Table 1. Classification of plant disease resistance genes (Hammond-Kosack and Jones, 1997)**

<table>
<thead>
<tr>
<th>Class</th>
<th>Gene</th>
<th>Plant</th>
<th>Pathogen</th>
<th>Predicted features of R proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hml</td>
<td>Maize</td>
<td><em>Helminthosporium maydis</em></td>
<td>Detoxifying enzyme HC-toxin reductase</td>
</tr>
<tr>
<td>2</td>
<td>Pto</td>
<td>Tomato</td>
<td><em>Pseudomonas syringae (avrPto)</em></td>
<td>Intracellular ser/thr protein kinase</td>
</tr>
<tr>
<td>3a</td>
<td><strong>RPS2</strong></td>
<td><em>Arabidopsis</em></td>
<td><em>Pseudomonas syringae (avrRpt2)</em></td>
<td>CC/NBS/LRR</td>
</tr>
<tr>
<td></td>
<td><strong>RPM1</strong></td>
<td><em>Arabidopsis</em></td>
<td><em>Pseudomonas syringae (avrRpm1/avrB)</em></td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td><strong>N</strong></td>
<td>Tobacco</td>
<td>Tobacco mosaic virus</td>
<td>TIR / NBS / LRR</td>
</tr>
<tr>
<td>4</td>
<td><strong>L6</strong></td>
<td>Flax</td>
<td><em>Melampsora lini</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>RPP5</strong></td>
<td><em>Arabidopsis</em></td>
<td><em>Peronospora parasitica</em></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><strong>Cf-2</strong></td>
<td>Tomato</td>
<td><em>Cladosporium fulvum</em></td>
<td>Extracellular LRR</td>
</tr>
<tr>
<td></td>
<td><strong>Cf-4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Cf-5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Cf-9</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><strong>Xa21</strong></td>
<td>Rice</td>
<td><em>Xanthomonas oryzae</em></td>
<td>Extracellular LRR/kinase domain</td>
</tr>
</tbody>
</table>
Classical genetic and molecular data show that plant R genes are frequently clustered in the genome (Michelmore and Meyers, 1998). The well-known mechanisms for generating diversity in R genes are duplication and recombination and molecular evidence has been generated to support this model (Lehmann, 2002). The ability of plant species to generate useful diversity at R gene loci would determine the plant's capability to ward off attack by newly evolving pathogen strains (Lehmann, 2002).

**Subcellular localization of R proteins**

In recent years, a number of R-Avr systems have been studied through genetic and molecular analysis. An important and interesting feature that has emerged is the localization of R proteins that are the products of R genes. The functions of these proteins appear to be dependent on their subcellular localization. These proteins have been found in a variety of cellular locations depending on the localization of the eliciting pathogen or its Avr determinant (Burch-Smith et al., 2007). For example, Cf proteins of tomato which are involved in conferring resistance to tomato against *Cladosporium fulvum* are localized in the plasma membrane (Rivas and Thomas, 2003). *Arabidopsis* RPM1 and RPS2 are found in the cellular membrane together with their corresponding Avr elicitors AvrRpm1 and AvrRpt2 (Axtell and Staskawicz, 2003). R proteins may also be found in the nucleus of plant cells as in the case of *Arabidopsis* RRS1-R in the presence of its bacterial Avr elicitor PopP2 (Deslandes et al., 2003). Many NB-LRR proteins do not carry subcellular targeting signatures and hence are believed to be cytoplasmic. However, cytoplasmic localization has been demonstrated only in two cases viz. *Solanaceae* R protein Bs2 (Leister et al., 2005) and barley protein Mla1 (Birr et al., 2004). A novel role for the TIR domain of R-proteins in association with pathogen-derived elicitors has been recently demonstrated in the interaction between tobacco N protein (product of tobacco N TIR-NB-LRR R gene that confers resistance to tobacco mosaic virus) and TMV elicitor p50 (Burch-Smith et al., 2007). This differs from the current models for plant innate immunity which propose that detection of Avr proteins is mediated solely through the LRR domain of R proteins. These studies show that N and p50 associate in the cytoplasm and nucleus of tobacco cells. This report also provides evidence for the first time for an in vivo association between an R protein and its corresponding Avr protein.

**Deploying R genes for generating durable disease resistance in plants through transgenesis**

It is now being increasingly possible to clone R genes from a wide variety of crops or their wild relatives as well as rapidly transfer them into elite cultivars. There are several instances where single R genes have been genetically engineered into crop plants. *Bs2* gene has been cloned from pepper (*Capsicum annuum*) and expression of this gene in tomato has been shown to confer resistance to bacterial spot disease caused by *Xanthomonas campestris* in tomato (Tai et al., 1999). The barley *Rpg1* gene has been shown to provide durable resistance to stem rust (Horvath et al., 2003; Brueggeman et al., 2002). Two tomato genes *Ve1* and *Ve2* (Kawchuk et al., 2001) are able to confer resistance to different *Verticillium* species and are functional in potato when expressed as transgenes (McDowell and Woffenden, 2003). However, the probability of durability of resistance of a single R gene remains since variations in the corresponding Avr genes can lead to lack of recognition of the pathogen (McDowell and Woffenden, 2003). This could be overcome to some extent by pyramiding multiple R genes into a single plant line.

Studies on non-host resistance could also provide clues to more useful genes which could confer uniform resistance to many pathogens (Heath, 2000). Non-host resistance refers to interactions in which all varieties of a plant species are resistant to all strains of a particular pathogen species. This is different from the
intraspecific variability observed for R-gene mediated resistance. As an example, *Arabidopsis* and tobacco are uniformly resistant to *Phytophthora infestans* – a pathogen that affects potato with devastating consequences. It has been recently shown that certain signal transduction components are involved in some non-host resistance interactions (Parker et al., 1996; Peart et al., 2002). Thus, one strategy that could be explored is to identify effective resistance genes against crop pathogens from model species and then transfer them to target crops.

Successful transfer of R genes from model species to target crops could be hampered by a phenomenon termed “restricted taxonomic functionality” – RTF (McDowell and Woffenden, 2003). According to this, R genes from one species can function as transgenes only within related species from the same family (Tai et al., 1999). For example, *Bs2* gene from pepper functions only in tomato, potato and tobacco and not in *Arabidopsis*. Conversely, *Arabidopsis RPS2* gene does not confer resistance in tomato (McDowell and Woffenden, 2003). However, this could be an useful strategy for increasing the breadth of potential resistance in at least a few genera or species (McDowell and Woffenden, 2003).

### Broad-spectrum resistance

An alternate strategy has been suggested for engineering broad-spectrum resistance in plants (De Wit, 1992) as many R genes have a narrow range of resistance. In this, a pathogen *Avr* gene is expressed in plant cells as a transgene under the control of a plant promoter that is induced by a range of pathogens. A corresponding R gene is also expressed (either an endogenous gene or a transgene). Upon pathogen attack, the pathogen-responsive promoter is activated, the *Avr* gene is expressed and the Avr protein interacts with the R protein to induce HR and other defence responses. One critical parameter in this strategy is the choice of the right promoter. The ideal promoter should not only respond to a wide variety of pathogens but also be inactive under disease-free conditions. Synthetic promoters have been designed to meet these requirements (Rushton et al., 2002). Broad-spectrum resistance could also be achieved through manipulation of defence-signalling components that act downstream of pathogen recognition (McDowell and Woffenden, 2003). However, care should be taken to avoid undesired side-effects that could manifest when defence responses are expressed constitutively as a result of such manipulations. The *Arabidopsis NPRI* gene has emerged as a good candidate gene for providing broad spectrum resistance. *NPRI* has been shown to regulate defence gene transcription (Fan and Dong, 1998). Enhanced resistance to diverse pathogens has been shown to be provided by over-expression of *NPRI* gene in *Arabidopsis* (Cao and Dong, 1998; Friedrich et al., 2001) and rice (Chern et al., 2001).

Induction of hypersensitive response (HR) results in programmed cell death (PCD), which is an effective defence against biotrophs and not against necrotrophs. Recent studies have shown that resistance to necrotrophs could be achieved by using mammalian genes that encode anti-apoptotic proteins (Dickman et al., 2001).

### R proteins as monitors

The classical gene-for-gene hypothesis suggests that R genes respond specifically to the direct or indirect products of cognate genes in pathogens and predicts a direct interaction between the R protein and the corresponding Avr protein (Flor, 1956, 1971). However, a direct interaction has been shown only for a few R-Avr pairs so far (Burch-Smith et al., 2007; Deslandes et al., 2003; Dodds et al., 2006; Scofield et al., 1996; Tang et al., 1996; Jia et al., 2000; Ueda et al., 2006). An explanation for the lack of direct interaction has been provided by suggesting an alternate role for the R proteins as ‘guard’ proteins. According to the ‘guard hypothesis’
proposed by Van der Biezen and Jones (Van der Biezen and Jones, 1998), the Avr protein induces a change in a host protein targeted by the pathogen to establish a successful infection and this change is sensed by the R protein, which acts as a guard, leading to the activation of the R protein and subsequent defence signalling. This model for R protein activation is supported by evidence from Arabidopsis R proteins RPM and RPS2 and host protein RIN 4 (Mackey et al., 2002), tomato Clt2 and host protease Rcr3 (Rooney et al., 2005) and Arabidopsis RPS5 and PBS1 (Shao et al., 2003). Thus, the guard model represents a significant shift in the modus operandi of R proteins from acting as passive security guards that wait for specific signals from an invader to an active role in continuously monitoring key physiological processes that are targeted by pathogens (McDowell and Woffenden, 2003). This different role for the R proteins also has implications for engineering resistance. It might be possible to extend the range of R gene functionality through transgenic transfer of guard-guardee pairs. One could also even think of altering the guardee proteins to make them less susceptible to pathogen targeting without affecting the guardee’s endogenous function, which is, of course, a challenge (McDowell and Woffenden, 2003).

CONCLUSIONS
A lot of knowledge has been generated at the molecular level on host-pathogen interactions. A host of R genes have been cloned, characterized and their functions elucidated. However, several gaps remain in our overall understanding of the entire process of plant defence. The entire network of plant defence signalling appears to be both intriguing and complicated and we need to increase our knowledge base on these aspects before we can devise rational strategies to genetically engineer durable resistance in crop plants. The rapid strides being made in functional genomics would definitely help in providing new insights into plant-pathogen interactions at the molecular level. More studies also need to be carried out at the protein level on the structural basis of recognition between R gene and Avr gene products. The constant evolutionary tug-of-war between plants and pathogens would nevertheless continue and hence it would be difficult to visualize an ideal panacea in terms of introducing durable, broad-spectrum resistance in plants against pathogen attack. However, considering the pace at which our knowledge on the basic aspects of plant-pathogen interactions is growing and given the advanced molecular tools available, the day is not far off when we would be able to design and use specific R genes to overcome major diseases affecting crop plants.

REFERENCES


