Abstract

Resistant genes are major regulators that confer resistance against specific pest or pathogen to crop plants. Understanding the resistance mechanism and characterization of resistant (R) genes in wild species often called as crop wild relatives (CWR), offers scope for developing pre-breeding stocks as well as evolving resistant varieties. In this context, the first and foremost step is to understand the structure and function of R genes. This article provides insights into the structure and function of R genes with special reference to pathogens of different agricultural crop plants. Both classical and latest examples of characteristic features of R genes and the mechanism of action are briefly described here.
Introduction

Identification of resistant gene pools against specific pest and disease is an important factor that facilitates faster and efficient breeding of resistant varieties in different crop plants. Most importantly the resistance (R) genes for any stress (biotic or abiotic) are reported to be present in crop wild relatives (CWR) or wild species of the crop plants (St. Clair, 2010; Kou and Wang, 2010). Resistance trait is considered as a dominant or recessive one which may be either monogenic or polygenic in inheritance. When the resistance trait is governed by polygenes they are referred as Quantitative trait loci (QTLs) and are located on the same chromosome or different chromosomes. Sometimes, this trait is also governed by a single gene and is called monogenic trait as observed with pvr3 gene conferring resistance to pepper–Potato virus Y (Palloix et al., 2009). The genes present in the resistant plant species, variety and/or cultivar confers resistance against specific pests or pathogens through different mechanisms involving stress signaling, pathogenesis activated molecular patterning, host defense proteins, reactive active species scavenging enzyme systems etc (Anderson et al., 2018). A specific resistant plant species may possess any one or combination of more than 2 mechanisms that make it as a resistant gene pool. Based on the number of resistance mechanisms, resistance levels are defined to be moderately resistant, resistant and highly resistant genotypes for a specific pest or disease in a specific crop plant. For instance, Elaichi is a resistant cultivar for mango malformation while Beauty Mclein is considered as susceptible genotype. QTLs contribute for the durable resistance and thus characterization of R genes in the CWRs is crucial for resistance breeding. Therefore, understanding the structure and function of R genes would be an important approach in crop improvement and this paper details with such information focusing on classical and latest examples.

Classification of R genes:

R genes have been characterized in many crops against specific pathogens/pests and are often referred as RGAs (Resistant gene analogs). On the basis of presence of specific domains in the protein encoded by these R genes, they can be broadly
classified into 5 distinct classes, viz., CNL, TNL, RLP, RLK and miscellaneous. However, some literatures cites 14 subcategories based on the presence or absence of certain domains but the broad classification is major 5 classes of R genes which are discussed subsequently (Table 1).

1. CNL class: CLN class R genes encode cytoplasmic proteins consisting of 3 main domains, coiled-coil domain, followed by nucleotide binding site domain and a leucine-rich repeat (CC-NBS-LRR). Example I2 gene of tomato which provides resistance against *Fusarium oxysporum* (Giannakopoulou et al., 2015).

2. TNL class: This class of R genes is characterized by the presence of N-terminal domain showing homology with mammalian Toll-interleukin receptor-like (TIR) and two other domains such as nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains. N, RPP5, and L6 are few examples falling in TNL class of R genes (Whitham et al., 1996).

3. RLP class: RLP class of R genes encodes for a receptor protein with intracellular Serine-Threonine kinaselike domain, and an extracellular leucine-rich repeat (LRR) domain. eLLR does not involve in pathogen recognition but they play an important role in action of many defense proteins. Polygalacturonase inhibiting proteins (PGIPs) are one of the classical examples of such proteins. Similarly, RLP class of R genes viz. Cf-2, Cf-4, Cf-9 and Cf12 genes have been found in tomato which provide resistance against leaf mold caused by *Cladosporium fulvum* (Xue et al., 2017).

4. RLK class: RLK class of R gene encodes protein which contains an intracellular kinase domain, and an extracellular leucine-rich repeat. *Xa21* is an example of RLK class which is responsible for resistance against Xantomonas in rice (Peng et al., 2015).

5. Miscellaneous class: This class of genes confers resistance in host plants through different molecular mechanisms, e.g. *Mlo* and *Asc-1. Mlo* gene encodes for 533 amino acid containing protein of size 60kDa. This gene is an example for monogenic, recessive nature of resistance against Barley powdery mildew pathogen, *Blumeriagraminis* sp. *hordei*.
### Table 1: Examples of R genes characterized in different crop plants

<table>
<thead>
<tr>
<th>Crop</th>
<th>Disease</th>
<th>Pathogen</th>
<th>R Gene</th>
<th>Resistant protein</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Maize</td>
<td>Leaf blight and Ear mold disease</td>
<td><em>Cochliobolus carbonum</em> race 1</td>
<td>HM1</td>
<td>NADPH-dependent HC toxin reductase (HCTR)</td>
<td>Johal, G., &amp; Briggs, S. (1992)</td>
</tr>
<tr>
<td>Tomato</td>
<td>Leaf speck disease</td>
<td><em>Pseudomonas syringae</em> pv. <em>tomato</em></td>
<td>Pto and Prf</td>
<td>NBS-LRR</td>
<td>Mysore <em>et al.</em> (2002)</td>
</tr>
<tr>
<td></td>
<td>Late blight, Wilt</td>
<td><em>Phytophthora infestans</em>, and <em>Fusarium oxysporum</em> f. sp. <em>lycopersici</em></td>
<td>I2</td>
<td>NLR</td>
<td>Giannakopoulou <em>et al.</em> (2015)</td>
</tr>
<tr>
<td></td>
<td>Leaf mold disease</td>
<td><em>Cladosporium fulvum</em></td>
<td>Cf 4, Cf9 and Cf 12</td>
<td>LRR, PR1</td>
<td>Kruij <em>et al.</em> (2005), Xue <em>et al.</em> (2017)</td>
</tr>
<tr>
<td>Tobacco</td>
<td>Tobacco mosaic virus</td>
<td>Tobacco mosaic virus</td>
<td>N</td>
<td>TIR-NBS-LRR</td>
<td>Whitham <em>et al.</em> (1996)</td>
</tr>
</tbody>
</table>

**Location and Structure of R gene products (proteins):**

The five major classes of R gene products i.e., proteins are generally localized either extracellular or intercellular and possess specific domains (Fig.1) as discussed earlier in R gene classification. LRR and LRR-Kinase class of proteins are transmembrane in nature while the Kinase, TIR-NBS-LRR and CC-NBS-LRR proteins are intracellular in nature. The transmembrane proteins are receptor proteins and the intracellular proteins are the secondary messengers which are involved in the signal transduction pathway that allows induced resistance in host plants. Among the five classes of R-genes/proteins, NBS-LRR class is the major class which consists of NBS-LRR domains and constitute the major resistant genes in many crop plants for different pathogens conferring disease resistance.
Generally, NB domain binds either ATP/ADP or GTP/GDP and LRR domain is involved in protein-protein interactions as well as ligand binding. NBS-LRR genes can be further subdivided into toll interleukin 1 receptor (TIR-NB-LRR) and coiled-coil (CC-NB-LRR). NBS region forms the N-terminal and is made of three components, ATP binding region, kinase 2 and kinase 3a. The LRR region is the least conserved segment of the protein which forms the β-strand of the protein.

**Mechanism of action of R genes:**

Pathogen invasion is recognized by Pathogen Recognition Receptors (PRRs) which are generally composed of LRRs. LRRs are capable of recognition of fungal, bacterial, and virulent (nucleic acids) pathogens. Brassinosteroid insensitive 1 – associated receptor kinase (BAK1) is a key protein which provide PRRs ability to recognize the pathogen. After recognition of pathogen PRRs release kinases into nucleus which triggers the reprogramming of transcription. Wall associated kinase (WAKs), Pathogen-associated molecular pattern (PAMPs), and damage-associated molecular pattern (DAMPs) get activated and generate signal molecules by pectin and lectins degradation upon pathogen invasion.
R genes in plant genomes confer pathogen derived or induced resistance described as PDR. These genes encode for and produce R proteins which help in recognition of proteins produced by pathogen’s specific Avr genes. Rapid evolution of plant immune system played an important role in diversification of different R genes. In researches it was found, the R gene encoded proteins have modular domain structures and function by various dynamic interactions between specific domains. To combat with pathogen, plants activate innate immune response which is signaled by complex of these proteins with Avr proteins. AVR protein-R gene interactions results in resistance. The AVR proteins have been well characterized in case of bacterial pathogens but not in fungi, and oomycetes (Table-2).

Table 2: Avr-R gene interactions in host plant resistance

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pathogen</th>
<th>Host</th>
<th>R gene</th>
<th>Avr-gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Cucumber mosaic virus</td>
<td><em>Arabidopsis thaliana</em></td>
<td><em>RCY1</em></td>
<td>Coat protein</td>
<td>Takahashi <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>4.</td>
<td>Xanthomonas campestris</td>
<td><em>Capsicum annuum</em></td>
<td><em>Bs2</em></td>
<td>Avr-Bs2</td>
<td>Tai <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>5.</td>
<td>Puccinia graminis.sp.tritici</td>
<td><em>Hordeumvulgarae</em></td>
<td><em>Rpg5</em></td>
<td>Avr-Rpg1</td>
<td>Kleinhofs(2009)</td>
</tr>
</tbody>
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Conclusion:
Under the modern Omics era, understanding the molecular mechanism conferring resistance in CWR through genomics, transcriptomics and proteomics tools also facilitates characterization of R genes and their expression products. R gene and Avr gene products could be studied using these approaches which would facilitate better comprehension of the host plant resistance. Expressed transcripts of R genes could be mapped onto the quantitative trait loci (QTL) governing disease resistance though expression-QTL (eQTL) mapping and
co-localization analysis. Perhaps, these molecular tools would not only increase the efficiency of resistant breeding but also reduce the time in developing new varieties and hybrids in crop plants.

References:


