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Plant Pattern Recognition Receptors: Biotechnological approach for broad spectrum disease resistance in crops

Kumari Surbhi*, Pradeep Negi, Kavita Negi
Department of Plant Pathology, College of Agriculture,
Govind Ballabh Pant University of Agriculture and Technology, Pantnagar

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Introduction

The primary purpose of agricultural operations is to produce enough food grains to provide food security in the country. Plant diseases are one of the most significant obstacles in realizing full yield potential of the crop. In nature, plants and their pathogens have co-evolved in time. In spite of the existence of so many pathogenic microbes in their vicinity, the plants usually do not become sick and have innate ability to ward off infection by pathogenic organisms. Thus, resistance is the rule and disease is an exception. Disease resistance refers to the ability of a plant to partially or completely, exclude or overcome the effect of a pathogen or other damaging factor (Agrios, 2005). The plant varieties which possess the properties to hinder the development of a given pathogenic organism, suffering little or no infection, are termed as resistant. The resistance of plants to pathogens could be divided into two fine categories i.e. host-resistance and non-host resistance. Host resistance refers to the resistance conferred by a single or a few related genes denoted as R genes. These genes are responsible for host-pathogen specificity. For each gene that confers virulence to the pathogen there is a corresponding gene that confers resistance in the host. This resistance may also be referred as race-cultivar specific resistance. The product of avirulence gene (*avr* gene) of the pathogen i.e. elicitor, brings about the recognition of pathogen by the host and subsequent activation of defense responses in the plant. Non host resistance refers to the resistance of an entire plant species to all the genetic variants of a pathogen. In non-host resistance, specific gene for gene interaction is not present and as such the pathogen lacking the gene for virulence could not infect a plant species (Lee *et al.*, 2016).

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Pathogen associated molecular patterns (PAMP): PAMPs also referred to as microbe associated molecular patterns are conserved molecules which are characteristic of a particular class of microbes. These PAMPs are recognized by plants with the help of receptor kinases present in their plasma membrane (Boutrot and Zipfel, 2017).

Damage associated molecular patterns (DAMPs): These are the molecules released as a result of pathogen attack or damage of plant cell. These DAMPs include fragments of cell wall or proteins, amino acids, nucleotides, cytosolic proteins which may be released from damaged cells. They also include oligomeric pieces of plant cell-wall polysaccharides that are released when tissues are disturbed by physical damage, infections, or herbivore attacks (Hou *et al.*, 2019).

Plant pattern recognition receptors

Plants have certain pattern recognition receptors (PRRs) which are localized on their cell surface. These PRRs may be of two kinds i.e. receptor kinases (RKs) or receptor-like proteins (RLPs). An ectodomain for ligand binding, a transmembrane domain, and a kinase domain make up receptor kinases whereas there are no clear intracellular signalling regions in the receptor-like proteins (RLPs) (Boutrot and Zipfel, 2017). In addition to these, plants also possess nucleotide binding site leucine rich repeat receptors (NBS- LRRs). The PRRs detect the presence of apoplastic elicitors and NBS- LRRs detect the cytoplasmic effectors delivered into the host cell by the pathogen. The PRRs are capable of perceiving both PAMPs and DAMPs. The recognition of ligand by the PRRs and subsequent signal transduction for defense in the plant is termed as pattern or PRR- triggered immunity (PTI). Through the production of both local and systemic immune responses, it contributes to both basal immunity to adapted pathogens and non-host resistance to non-adapted pathogens.

Pattern triggered immunity (PTI) and Effector triggered immunity (ETI)

Plants possess two major mechanisms for defending themselves against pathogens i.e. pattern recognition receptors (PRR) that recognise pathogen associated molecular patterns (PAMPs) and effector-triggered immunity (ETI).

- **Pattern triggered immunity (PTI):** It is a characteristic of non-host resistance. It Usually activates multilayered basal resistance mechanisms, such as peroxisome-based biosynthesis, pathogen growth restriction due to nutrient limitation, papilla development, and callose and lignin deposition.

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➢ **Effector triggered immunity (ETI):** It is the key mechanism of host-resistance. R-proteins recognise complementary pathogen effector proteins directly or indirectly in this host. A hypersensitive response (HR) including cell death often occurs as a result of the specific detection in the host plant, reducing or stopping disease progress.

Examples of PAMP and PRR interaction

➢ **FLS2 and flg22:** Distinct PRRs from plants and animals identify different flagellin epitopes. Flg22 is a 22-amino-acid peptide with a conserved N-terminus that acts as an elicitor. Flagellin binds to residues 9 to 15 in FLS2's LRR. FLS2 is a leucine-rich repeat receptor-like kinase found in Arabidopsis (LRR-RLK). It is necessary for the detection of bacterial flagellin and the activation of defensive responses, particularly via the MAPK cascade.

➢ **Mechanism of resistance in rice to bacterial blight (*Xanthomonas oryzae* pv. *Oryzae*):** Rice varieties with the Xa21 gene possess broad-spectrum disease resistance to *Xanthomonas oryzae* pv. *Oryzae* strains with the corresponding Ax21 gene which activates the XA21-mediated immunity in rice. Components of Xa21 include extracellular LRR domain, transmembrane domain, juxtamembrane domain, and kinase domain. African wild rice, O. longistaminata, was used to isolate the Xa21 gene.

➢ **Recognition of Chitin:** *Cladosporium fulvum*, the tomato leaf mould fungus, enters the host through stomatal pores and develop as extracellular hyphae. The fungus cloaks its chitin-containing cell walls with the chitin-binding effector Avr4 to protect them from the activity of chitin-degrading enzymes. The Tomato RLP Cf-4 can detect the presence of Avr4, resulting in the development of the hypersensitive response. In Arabidopsis, the RLK CERK1 and in rice, both OsCERK1 and the RLP OsCEBiP identify chitin oligomers produced by chitinases as PAMPs. To avoid chitin-induced PTI, *Cladosporium fulvum* can secrete Ecp6, a chitin-scavenger effector that removes chitin oligomers produced by chitinases.

Identification of plant pattern recognition receptors (PRRs)

RKs and RLPs are used by plants as PRRs to detect apoplastic elicitors (PAMPs or DAMPs). Functional evaluation of known compounds, biochemistry, sequencing and genome analysis have all been used to identify PAMPs and DAMPs while the PRRs have primarily been discovered through genetic and biochemical methods.

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(A) Genetic Approaches: Identification methods of PRRs include forward and reverse genetics approaches.

➢ **Forward genetics:** The aim of forward genetic studies is the identification of the gene that causes a specific phenotypic trait. The various approaches used under Forward genetics include mutational breeding (induction of random mutations and subsequent mapping of genes), linkage mapping, map-based cloning and insertional mutagenesis (with the use of a well-known T-DNA or transposon tag) (Boutrot and Zipfel, 2017).

➢ **Reverse genetics:** In reverse genetics typically, a single gene is targeted and its function is altered to evaluate its expression i.e. its phenotypic effect. The approaches used under reverse genetics include RNA interefrence (silencing of target gene expression), anti sense RNA (blocking of translation of mRNA), over-expression of gene with the use of a strong promoter, site directed mutagenesis (TILLING), Genome editing (CRISPR/Cas) and analysis of transcriptome (Boutrot and Zipfel, 2017).

(B) Biochemical Approaches: Using insoluble or immobilised ligands for affinity purification of PRRs via affinity chromatography, the PRRs may be identified. Using their co-receptors as molecular bait is another biological way of identifying PRRs.

Use of PRRs in genetic engineering for broad spectrum disease resistance in crops

PRRs can be used to boost plant immunity against adapted pathogens in a variety of plant species. PRRs can be genetically modified to improve ligand recognition and intracellular immune responses. The use of transgenes from some unrelated and heterologous species of plants may present a strong strategy for improving the long term resistance of economically important crops to different fungal and bacterial pathogens. Individual PRR genes are considered to offer wide resistance against numerous infections since PAMP molecules are conserved among microbial species. Adapted pathogens, on the other hand, may generate PAMPs that bypass plant PRR-mediated sensing by changing critical residues implicated in PRR-PAMP interactions. Furthermore, the interfamily transfer of a new PRR to a specific plant species may increase PTI responses by signalling more PTI activation through the new PAMP/PRR recognition system. Pathogen PAMPs that are specialised to a specific host find it more difficult to overcome the new non-host PRR signalling resistance pathways. Signaling networks downstream of PRRs are highly conserved, not only within but also between plant families and even monocot and dicot classes which permit functional

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transfer of PRRs between. This makes PRRs particularly interesting targets for genetic engineering of plant immunity. PRRs being the apparatus for ligand binding and defining epitope specificity of receptors in pattern triggered immunity, the transfer of a PRR with unique epitope specificity could render the power of recognition of epitopes in plant species which were earlier insensitive to the pathogen (Boutrot and Zipfel, 2017; Pfeilmeier, 2017).

The methods of transfer of PRRs include inter and intra-species transfer, inter-family transfer. Besides transferring the naturally occuring PRRs, new PRRs could also be engineered as per requirement through biotechnological approaches. There are several examples of successful transfer of PRRs and improved resistance of plant species to pathogen. The EFR receptor which is specific to the plant family *Brassicaceae*, has been used to develop transgenic tomato and tobacco plants which show improved sensitivity to bacterial pathogens carrying elf18 epitope. This EFR gene has also been transferred to monocot crop plants including wheat and rice. Some other genes transferred under this strategy include Xa21 from rice (detects the RaxX protein from *Xanthomonas oryzae pv*. *Oryzae*) and the receptor ELR from wild potato (provides resistance to (*Phytophthora infestans*). Endogenous PRRs can be improved, or novel, chimeric PRRs can be introduced. This provides plants with greater MAMP/DAMP sensing and signalling capabilities, resulting in increased PTI against a wide range of invaders (Boutrot and Zipfel, 2017).

One major concern related to the introduction of novel PRRs into plant species is that it may raise the possibility of disrupting beneficial relationships between these plants and their natural microbiota or symbiotic associations, such as legume-rhizobia or mycorrhizal relationships. Adapted symbionts may have developed effective methods to escape or repress host PTI at different levels.

Conclusion and Future prospects

Classic breeding procedures can be used to introduce relevant PRR genes from wild relatives. Well beyond restrictions of sexual compatibility, advanced genetic engineering technologies enable direct transfer of PRR genes between plant families. This also enables the use of nearly any plant species as a resource of PRR genes. As a result, PRR transfer has a lot of promise for conferring broad-spectrum and possibly long-lasting resistance characteristics to crop plants. These genetic tools offer a high scope for devoloping sustainable plant disease management techniques.