Resistant Gene Analog (RGA) in Plant Disease Management

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Abstract

Plants are susceptible to numerous biotic and abiotic stresses during their life cycle. Abiotic stress includes high or low temperature, drought, floods and biotic stresses include diseases caused by fungi, bacteria, viruses, nematodes, mites and insects. Plant immune system has evolved into a specific mode by years of co-evolution and host-microbe interactions resulting in enormous complexity at both the cellular and molecular levels. Plants have developed effective mechanisms mediated by R-genes which activate strong immune defence. Resistance genes (R-genes) are very valuable resources to better understand plant defence mechanism and develop disease resistant varieties or hybrids. Resistant Gene Analogues in plants are large class of potential R-genes which have conserved domains and motifs related to the NBS-LRR, receptor like kinases, receptor like proteins with their structural features. These conserved motifs are facilitating the use of RGA from distantly related plants. Those conserved natures of sequences help scientists to fish out resistance gene over species and genera. These sequences are used as probes to identify similar sequences in other plant, variety or species.

Introduction

Plant immune system is less complex than its animal counterpart and there are some fundamental differences between plants and animals. Somatic circulatory adaptive immune system and mobile immune cells are absent in plants. Plants are entirely dependent on their innate immune responses in each and every individual cell. Plant immune system has evolved into a specific mode by years of co-evolution and host-microbe interactions resulting in enormous complexity at both the cellular and molecular levels. The cells recognize systemic signals from the infection site and finally develop resistance responses to that particular pathogen. Genes in the plant genome that convey resistance against pathogen by producing R proteins are Resistance genes. These genes are very valuable resources to better understand plant defense mechanism and develop disease resistant varieties/hybrids. The main function of R-genes is initiation of plant defense-signaling mechanism and detection of plant-specific pathogen elicitors (Morel and Dangl, 1997). Kabi *et al.* (2017) studied the molecular analysis of twenty-six different green gram genotypes to link the available RGA markers with YMV resistance by using a resistant gene analog (RGA) marker named CYR1 which produced amplicon at 90 bp in seven genotypes. They concluded that seven genotypes had yellow mosaic virus resistance gene and the marker was an efficient and ubiquitous for genotyping of YMV reaction. OBGG 2013-20 was an YMV resistance and high yielding line which can be used as YMV donor or can be released as a variety. CYR1-RGA marker was completely linked with YMV resistance and would assist in identifying plants endowed with resistance locus conferring aid in the development of resistant cultivars in relatively shorter time span (Reddy and Naresh, 2018).

A study was carried on the cloning of RGAs from a genomically under-utilized crop, finger millet (*Eleusine coracana*) to identify functional disease resistance gene**s.** Comparison of finger millet RGA to sequences available in NCBI database revealed that it was most similar to NBS-LRR proteins from other plant species especially the cereal, rice. The greatest similarity was shown with RPS2 like disease of kinase 2 motif which is indicative of the non-TIR sub-class of R-genes, the prominent class of R-genes. Phylogenetic analysis of finger millet RGA with various classes of plant RGAs indicated that they formed a cluster with

RPS2 type NBS-LRR proteins and among them it was closer to rice. RPS2 type R-genes that were reported in other plant species were used for phylogenetic analysis and finger millet RGA formed a cluster together with *Oryza sativa* RPS2 protein indicating the phylogenetic closeness of rice (Jacob, 2017).

Resistance gene classification

Class	Function	Examples of R gene
1	Membrane associated, transcription regulating broad spectrum resistance	*RPW8*
2	Cytoplasmic signal transducing serine-threonine protein kinase	*PTO*
3	Extracellular LRRs with transmembrane anchor	*Cf-2 – Cf-9*
4	Extracellular LRRs, with a transmembrane receptor and a cytoplasmic serine-threonine kinase	*Xa21*
5	Cytoplasmic, membrane associated, Contain LRRs, NBS, and TIR domain	*RPP5, N1, L6*
6	Also cytoplasmic, membrane associated, contain LRRs, NBS, and a coiled coil domain	*RPM1, RPS2*

Zhou *et al.* (2019) validated 10 target RGAs in chickpea for their differential expression in response to *A. rabiei* infection. Assessed gene expression at each RGA locus via qPCR at 2, 6, and 24 h after *A. rabiei* inoculation with a previously characterized highly aggressive isolate. Among four varieties including two resistant cultivars (ICC3996 and PBA Seamer), one moderately resistant cultivar (PBA HatTrick) and one susceptible cultivar (Kyabra), RGAs differential expression was significant and consistently increased in the most resistant genotype ICC3996 immediately following inoculation. Thus, they showed that the RGAs are key factors in the recognition of plant pathogens and the signaling of inducible defenses. These represent clear targets for future functional validation and potential for selective resistance breeding for introgression into elite cultivars.

Intracellular signaling mechanisms of RGAs in plant defense

TNL and CNL proteins recognize pathogen effectors that are secreted into the cell allowing plants to trigger the ETI response. *RIN4, PBS1, Pto* and *EDS1* are targeted and modified by numerous effectors and, as a result, their corresponding TNL or CNL will detect the modification to initiate ETI responses. Aside from targeting immune regulatory components, effectors can also target PTI/MTI signaling cascades. MAP kinase cascade, specifically *MPK4,* is capable of suppressing NBS-LRR protein *SUMM2* in absence of effector *HopAl1*. TIR-TIR interactions occur between *RPS4* and *RRS1* to further activate defense genes. Flg22, a bacterial PAMP, activates *FLS2* and *BAK1* RLKs to initiate the MAP kinase cascade that triggers PTI/MTI responses. MAP kinase cascade signaling can be interrupted by pathogenic effectors. When *MPK4* is compromised, *SUMM2* will not be inactivated and will initiate PCD.

Conclusions

RGAs are an efficient tool in identifying and isolating the disease resistant genes and have got efficiency in building durable resistance. Knowledge on a clear, functional mechanism of plant-microbe interaction and downstream pathways of disease resistant genes is moderate. The new, genomic, high approaches like Next Generation Sequencing (NGS) and other techniques, can pave the way for a better understanding of resistance mechanism in plants to pathogens. Comprehensive information and knowledge on RGA and genomic R loci architecture will help to develop multiple-disease resistance varieties or hybrids.

References

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