Plant Science 270 (2018) 72–84

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review article

Review: Potential biotechnological assets related to plant immunity modulation applicable in engineering disease-resistant crops

Marilia Santos Silva[a,**,1], Fabrício Barbosa Monteiro Arraes[a,b,***,1], Magnólia de Araújo Campos[c], Maira Grossi-de-Sa[d], Diana Fernandez[d], Elizabete de Souza Cândido[e,f], Marlon Henrique Cardoso[e,f,g], Octávio Luiz Franco[e,f,g], Maria Fátima Grossi-de-Sa[a,b,e,g,*]

[a] Embrapa Recursos Genéticos e Biotecnologia (Embrapa Cenargen), Brasília, DF, Brazil
[b] Universidade Federal do Rio Grande do Sul (UFRGS), Post-Graduation Program in Molecular and Cellular Biology, Porto Alegre, RS, Brazil
[c] Universidade Federal de Campina Grande (UFCG), Center of Education and Health Cuité-PB, Brazil
[d] IRD, CIRAD, Univ. Montpellier, IPME, Montpellier, France
[e] Universidade Católica de Brasília (UCB), Post-Graduation Program in Genomic Science and Biotechnology, Brasília, DF, Brazil
[f] Universidade Católica Dom Bosco (UCDB), Campo Grande, MS, Brazil
[g] Universidade de Brasília (UnB), Brasilia, DF, Brazil

ARTICLE INFO

Keywords:
Pathogen-associated molecular pattern-triggered immunity (PTI)
Effector-triggered susceptibility (ETS)
Effector-triggered immunity (ETI)
RNA interference
Genetically modified (GM) crop
Disease resistance

ABSTRACT

This review emphasizes the biotechnological potential of molecules implicated in the different layers of plant immunity, including, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), effector-triggered susceptibility (ETS), and effector-triggered immunity (ETI) that can be applied in the development of disease-resistant genetically modified (GM) plants. These biomolecules are produced by pathogens (viruses, bacteria, fungi, oomycetes) or plants during their mutual interactions. Biomolecules involved in the first layers of plant immunity, PTI and ETS, include inhibitors of pathogen cell-wall-degrading enzymes (CWDEs), plant pattern recognition receptors (PRRs) and susceptibility (S) proteins, while the ETI-related biomolecules include plant resistance (R) proteins. The biomolecules involved in plant defense PTI/ETI responses described herein also include antimicrobial peptides (AMPs), pathogenesis-related (PR) proteins and ribosome-inhibiting proteins (RIPs), as well as enzymes involved in plant defensive secondary metabolite biosynthesis (phytoanticipins and phytoalexins). Moreover, the regulation of immunity by RNA interference (RNAi) in GM disease-resistant plants is also considered. Therefore, the present review does not cover all the classes of biomolecules involved in plant innate immunity that may be applied in the development of disease-resistant GM crops but instead highlights the most common strategies in the literature, as well as their advantages and disadvantages.

1. Introduction

Plant pathogens, including viruses, bacteria, fungi, and oomycetes are a primary concern in agribusiness [1–3]. The diseases caused by these organisms in plants represent an important and persistent threat to food supplies worldwide [4]. The development of disease-resistant plants through biotechnological approaches aims to obtain economically important crops through elite genetically modified (GM) lines that not only display durable and broad-spectrum resistance to multiple phytopathogens, but that are also biosafe to the environment and consumers. To achieve this goal, several challenges related to transgene must be overcome, such as fine-tuning the choice, origin (i.e., heterologous species and/or non-host plant) and the number of genes to be employed and stacked, as well as gene expression control (e.g., by signal peptides, gene silencing and gene promoters). The current knowledge of the molecular mechanisms involved in plant-pathogen interactions has now provided a large set of biomolecules that can be applied in the development of GM disease-resistant/less susceptible crops.

Plant-pathogen interactions involve a two-way communication process, whereby plants can recognize and induce defense strategies against pathogens, while pathogens can threaten plant functional

* Corresponding authors at: Embrapa Recursos Genéticos e Biotecnologia (Cenargen), Brasília, DF, Brazil.
** Corresponding author.
*** Corresponding author at: Universidade Federal do Rio Grande do Sul (UFRGS), Post-Graduation Program in Molecular and Cellular Biology, Porto Alegre, RS, Brazil.
E-mail addresses: marilia.silva@embrapa.br (M.S. Silva), fabricio.arraes@gmail.com (F.B.M. Arraes), fatima.grossi@embrapa.br (M.F. Grossi-de-Sa).
[1] These authors contributed equally.

https://doi.org/10.1016/j.plantsci.2018.02.013
Received 9 October 2017; Received in revised form 1 February 2018; Accepted 4 February 2018
Available online 14 February 2018

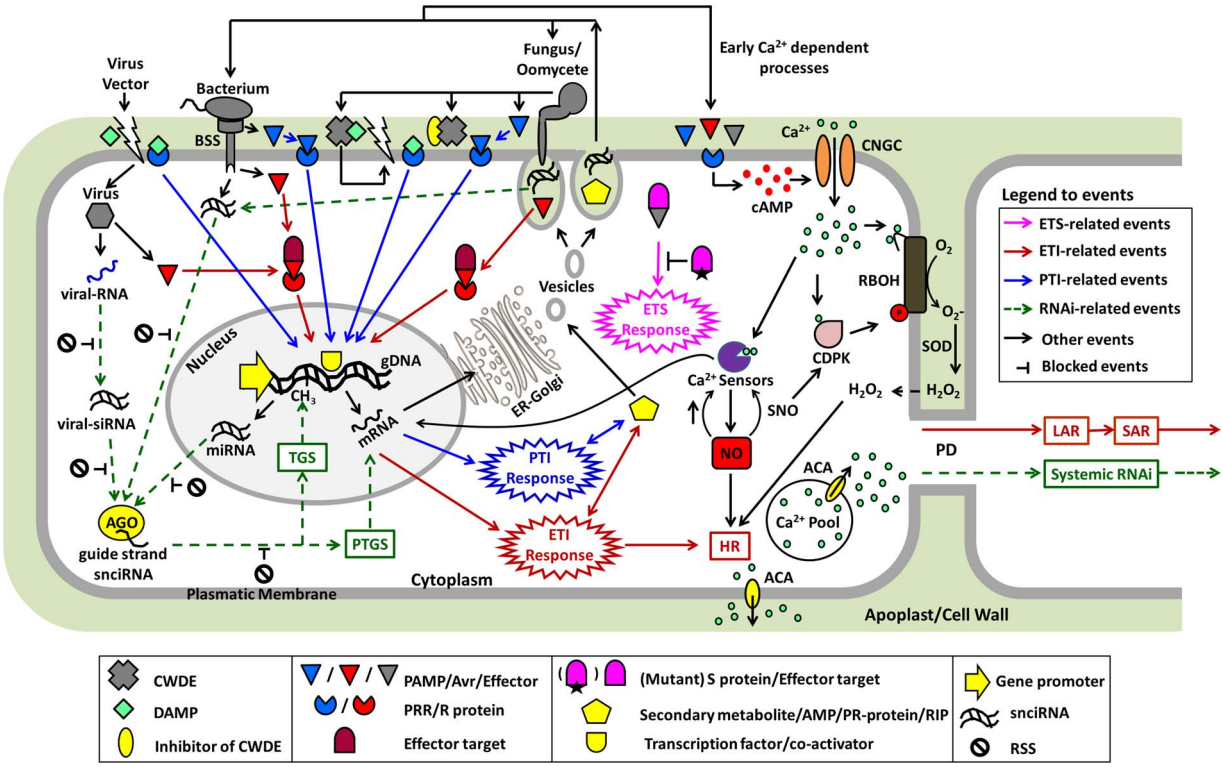

Fig. 1. General plant immune pathways and potential biotechnological assets found in plant-pathogen interactions used to engineering disease-resistant crops. The schematic figure illustrates the intricate relations between plant innate immunity (PTI, ETI), ETS-related resistance response and the regulation of the genes involved in plant-pathogen interactions by RNAi-mediated TGS and PTGS. Summary of manly early calcium (Ca^{2+}) dependent processes are also illustrated. The interaction of PAMP/Avr/Effectors with plasma membrane receptor induces cAMP production and stimulates the rapid influx of Ca^{2+} into the cell through CNGC. Free Ca^{2+} from apoplast and/or intracellular Ca^{2+} pools can stimulate H_2O_2 production by RBOH in two ways: (i) directly activation by Ca^{2+} interaction with RBOH N-terminal; (ii) and indirectly activation with RBOH phosphorylation by Ca^{2+} activated CDPK. Activated Ca^{2+} sensors (for example calmodulin/calmodulin-like) increase NO production, which regulates Ca^{2+} sensors by positive feedback and stimulates HR. Both Ca^{2+} sensors and CDPK are S-nitrosylated (SNO − a post-translational regulatory mechanism during which NO is covalently and reversibly bonded to the sulfahydryl groups of rare, low pKa cysteine residues). Ca^{2+} sensors can induce PTI/ETI through transcription regulation of genes related with stress responses. Intracellular Ca^{2+} levels can be regulated by the efflux of the second messenger through the ACA protein. For details and discussion, see text. Abbreviations (in alphabetical order): **ACA**: autoinhibited Ca^{2+} ATPase; **AGO**: argonaute; **AMP**: antimicrobial peptide; **BSS**: bacterial secretion systems; **cAMP**: cyclic AMP; **CDPK**: calcium-dependent protein kinase; **CH_3**: methyl; **CNGC**: cyclic nucleotide-gated channel; **CWDE**: cell wall-degrading enzymes; **DAMP**: damage-associated molecular pattern; **ER-Golgi**: endoplasmic reticulum-Golgi complex; **ETI**: effector-triggered immunity; **ETS**: effector-triggered susceptibility; **gDNA**: plant genomic DNA; **HR**: hypersensitivity response; **LAR**: local acquired resistance; **miRNA**: micro RNA; **mRNA**: messenger RNA; **NO**: nitric oxide; **PAMP**: pathogen associated molecular pattern; **PD**: plasmodesma; **PRR**: pattern recognition receptor; **PR protein**: pathogenesis-related protein; **PTGS**: post-transcriptional gene silencing; **PTI**: PAMP-triggered immunity; **R**: resistance protein; **RBOH**: NAPDH oxidase; **RIP**: ribosome-inhibiting proteins; **RSS**: RNA silencing suppressor; **S protein**: susceptibility protein; **SAR**: systemic acquired resistance; **siRNA**: small interfering RNA; **sncRNA**: small non-coding interfering RNA; **SNO**: S-nitrosylation; **SOD**: superoxide dismutase; **TGS**: transcriptional gene silencing.

physiology and counterattack plant defense mechanisms. The intricate plant-pathogen exchange of interactions among biomolecules involve specific characteristics depending upon whether the pathogen is a virus, bacterium, nematode or filamentous microbe: (i) viruses are directly introduced, either by mechanical damage or by a biological vector (i.e., insect, nematode, fungus) into the plant cell cytosol, where they expose their genome, structural proteins and lipids (in the rare case of enveloped viruses); (ii) bacteria biomolecules related to virulence are secreted by type II, III and IV secretion systems to interact with the host plant cell [5–7]; and (iii) filamentous pathogens (herein referred as Eumycota true fungi and oomycetes with fungal-like growth, also known as water molds) release a range of biomolecules into the plant apoplast and cytosol (Fig. 1). In opposition to the first barrier to plant invasion, filamentous pathogens secrete cell-wall-degrading enzymes (CWDEs) [8], and plants, in turn, respond to the cell wall damage by strengthening/reprogramming the cell wall and by secreting CWDE inhibitors.

Invasion by most pathogens is perceived through transmembrane plant proteins called pattern recognition receptors (PRRs), which detect microbe-derived molecules termed pathogen-associated molecular patterns (PAMPs). In addition to PAMPs, PAMP-triggered immunity (PTI) is also activated by endogenous plant signals released during pathogen invasion termed damage-associated molecular patterns (DAMPs). The first active line of plant immunity is triggered upon the specific detection of PAMPs by PRRs [9–11]. Well-adapted pathogens secrete a plethora of effectors (i.e., molecules secreted by pathogens that modulate host cell mechanisms and physiology) that suppress PTI through susceptibility (S) proteins (effector targets), allowing host cell infection and resulting in effector-triggered susceptibility (ETS) [12–15] (Fig. 1).

In response to effectors, plants developed a second line of receptors, encoded by resistance (*R*) genes, that are activated via specific recognition of the cognate effector or pathogen avirulence (Avr) proteins, yielding effector-triggered immunity (ETI) [16] (Fig. 1). PTI involves PAMPs that are evolutionarily conserved across a class of organisms, while ETI is highly specific to certain pathogens that secrete a unique effector or Avr product. ETI frequently involves localized programmed cell death, known as the hypersensitive response (HR) that restricts pathogen spread at the infection site [17]. To restrain infection, both PTI and ETI induce the expression of a range of antimicrobial peptides (AMPs), pathogenesis-related (PR) proteins, ribosome-inhibiting proteins (RIPs) and defensive secondary metabolites, among other plant physiological defense biomolecules [18–21]. The HR in infected cells is associated with the transfer of defense signals to neighboring uninfected cells within the same organ. This transfer is performed through plasmodesmata and to other uninfected organs through the phloem, which results in induced distal resistance responses called local acquired resistance (LAR) and systemic acquired resistance (SAR),