CellPress

Plant innate immunity – sunny side up?

Simon Stael[1,2,3,4], Przemyslaw Kmiecik[5], Patrick Willems[1,2,3,4], Katrien Van Der Kelen[1,2], Nuria S. Coll[6], Markus Teige[5,7], and Frank Van Breusegem[1,2]

[1] Department of Plant Systems Biology, VIB, 9052 Ghent, Belgium
[2] Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium
[3] Department of Medical Protein Research, VIB, 9000 Ghent, Belgium
[4] Department of Biochemistry, Ghent University, 9000 Ghent, Belgium
[5] Department of Ecogenomics and Systems Biology, Vienna University, Vienna, Austria
[6] Department of Molecular Genetics, Centre for Research in Agricultural Genomics, CSIC-IRTA-UAB-UB Consortium, 08193 Barcelona, Spain
[7] Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria

Reactive oxygen species (ROS)- and calcium- dependent signaling pathways play well-established roles during plant innate immunity. Chloroplasts host major biosynthetic pathways and have central roles in energy production, redox homeostasis, and retrograde signaling. However, the organelle's importance in immunity has been somehow overlooked. Recent findings suggest that the chloroplast also has an unanticipated function as a hub for ROS- and calcium-signaling that affects immunity responses at an early stage after pathogen attack. In this opinion article, we discuss a chloroplastic calcium-ROS signaling branch of plant innate immunity. We propose that this chloroplastic branch acts as a light-dependent rheostat that, through the production of ROS, influences the severity of the immune response.

ROS and calcium form the basic ingredients for plant innate immunity

Immunity is essential for plants to cope with pathogen infections, which would otherwise lead to dramatic losses in agriculture currently prevented through intensive and costly pest management strategies [1,2]. Upon infection, plant pathogenic fungi, oomycetes, viruses, and bacteria face the plant cell wall as a first barrier. When this barrier is breached, the pathogens are recognized by plant membrane pattern recognition receptors (PRRs) (see Glossary). These receptors can recognize microbial molecules, such as protein or cell wall derivatives that are often conserved in the same class of microbes, thereby activating pathogen- or microbial-associated molecular pattern (PAMP; MAMP)-triggered immunity (PTI; MTI) [3]. In order to inhibit PTI, pathogens can secrete virulence effector proteins into the plant cells, which in turn can be recognized by intracellular plant receptors of the nucleotide-binding and leucine-rich repeat domain class (NB-LRRs), thereby activating effector-triggered immunity (ETI). This second layer of

Corresponding author: Van Breusegem, F. (frank.vanbreusegem@psb.vib-ugent.be).
Keywords: chloroplast; calcium signaling; reactive oxygen species signaling; pathogen-triggered immunity; effector-triggered immunity; light; pathogen.

1360-1385/
 http://dx.doi.org/10.1016/j.tplants.2014.10.002

Glossary

Compatible versus incompatible interaction: compatible interactions take place in susceptible hosts, which are not able to recognize the pathogen effectors and therefore do not mount an ETI response. This leads to colonization of the host tissues. By contrast, incompatible interactions involve recognition of the pathogen effectors by the host's immune system, through direct or indirect interaction with NB-LRR proteins. This leads to ETI, which results in host resistance towards the pathogen, in most cases via an HR response.
Effector: proteins delivered by pathogens that modulate innate immunity and enable infection. Effectors can be secreted in the apoplastic space or into the cytoplasm of host cells through different secretion systems, where they target different subcellular compartments.
Effector-triggered immunity (ETI): a second layer of plant defense which is initiated by recognition of effector proteins by NB-LRRs. ETI is an amplified version of PTI that often results in the induction of the hypersensitive response (HR) cell death.
Hypersensitive response (HR): a plant-specific form of programmed cell death that typically accompanies and correlates with effector-triggered immunity (ETI) at the site of attempted pathogen invasion. HR is morphologically unique, involving cytoplasmic shrinkage, chromatin condensation, mitochondrial swelling, vacuolization and chloroplast disruption during its final stages.
NB-LRRs: plant intracellular receptors of the nucleotide-binding and leucine-rich repeat domain class (NB-LRR). NB-LRRs that can directly or indirectly recognize effector proteins secreted into the host cell, activating effector-triggered immunity (ETI). Also known as disease resistance (R) proteins.
PAMP/MAMP-triggered immunity (PTI/MTI): first layer of immunity activated by pathogen-associated molecular pattern (PAMP) recognition. Sometimes, this is also referred to as microbial-associated molecular pattern (MAMP) recognition. It involves a calcium burst, production of reactive oxygen species (ROS), callose deposition at the cell wall, activation of mitogen-activated protein kinase (MAPK) cascades, expression of defense-associated genes and production of ethylene.
Pathogen-associated molecular patterns (PAMPs): conserved molecules common to pathogens that can be recognized by immune receptors in both plants and animals.
Pattern recognition receptors (PRRs): host cell surface-localized receptors that can recognize PAMPs and initiate a signaling cascade leading to PAMP-triggered immunity (PTI).
Systemic acquired resistance (SAR): defense mechanism that confers long-term protection against a broad spectrum of pathogens to the whole plant following an earlier, localized exposure. SAR requires salicylic acid and is characterized by increased expression of pathogenesis-related genes both locally, at the initial site of infection, and systemically, in uninfected tissue.
Virulent versus avirulent: a pathogen is termed avirulent if it contains an effector protein that can be recognized directly or indirectly by the host's immune system (via NB-LRR proteins) resulting in resistance. By contrast, a pathogen is considered virulent if the host is not able to recognize any of its effectors, and is therefore able to cause disease. Therefore, a given pathogen can be virulent in a host plant that lacks the cognate NB-LRR proteins, but avirulent in another host plant that contains the NB-LRR proteins that lead to direct or indirect recognition of the pathogen effector(s).

immunity often culminates with a hypersensitive response (HR) programmed cell death [4]. Failure by the plant to recognize PAMPs or effector proteins paves the way for successful pathogen infection.

Over the past 3 decades, ROS have been well established as an integral aspect of plant immunity in a process generally described as the oxidative burst. This event was first described in 1983 upon infection of potato (*Solanum tuberosum)* tubers with an incompatible race of *Phytophtora infestans* [5]. Since then, numerous studies have described the nature, kinetics, and localization of ROS production, mostly indicating the apoplast as the hotspot of ROS production (reviewed in [6]). In 1990, the role of calcium as a secondary messenger in the immunity response emerged [7,8], making both ROS and calcium the first-responders-on-scene, together with apoplast alkalinization and the activation of kinase modules [9] (Figure 1A). Subsequent series of pharmacological and genetic perturbation studies consolidated an upfront role for calcium fluxes and the necessity of a downstream oxidative burst for plant defence (measured by pathogen growth) [10–12]. Cytoplasmic calcium levels are kept low by sequestering free calcium ions in the apoplast and diverse subcellular stores. Within 1 min, pathogen recognition leads to a reversible release and uptake of calcium to the cytoplasm through calcium channels and pumps, respectively [13]. Originally, screens for mutant plants exhibiting altered defence response led to the identification of three lines (*dnd1, defence no death 1; dnd2 / hml1, dnd2 / HR-like lesion mimic 1*; and *cpr22, constitutive expresser of PR genes22)* that are linked to Ca^{2+}-permeable cyclic nucleotide-gated channels (CGNC2, CGNC4, and CGNC11/12, respectively). In addition, ionotropic glutamate receptor-like channels (iGluR), and calcium ATPases have also been implicated in plant immunity (for an in-depth review of calcium pumps and channels, refer to [14]; also see Figure 1A). The calcium signatures produced are stimulus-specific and are decoded by calcium-dependent protein kinases (CDPKs), whose deficiency in turn leads to impaired defence responses [15–17]. Although NADPH

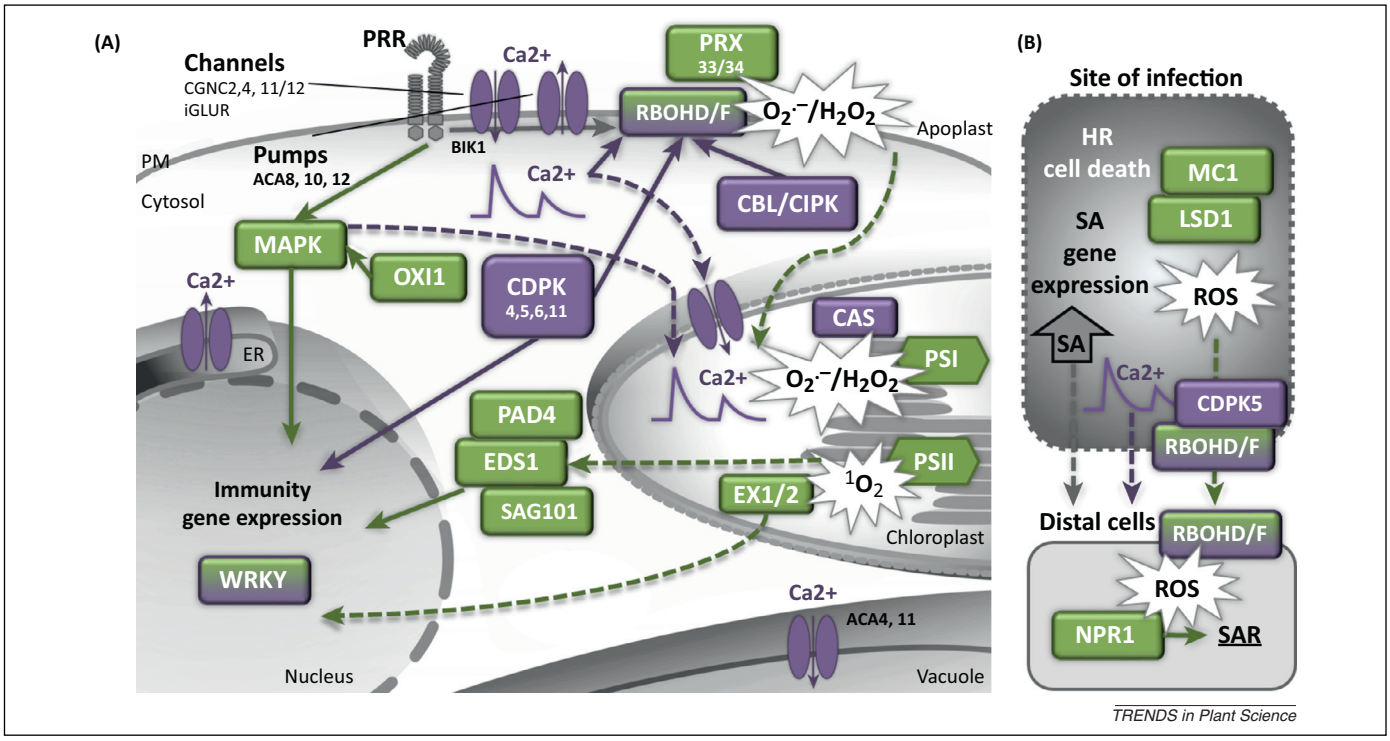

Figure 1. Reactive oxygen species (ROS) and calcium signaling pathways during plant immunity. **(A)** A fast response is triggered upon pathogen-associated molecular pattern (PAMP) perception by pattern recognition receptors (PRRs) signaling the concerted action of calcium (Ca^{2+}) channels and transporters that generate a cytosolic Ca^{2+} flux (within 5 min after elicitation). Calcium-dependent protein kinases (CDPKs), upon activation by the Ca^{2+} flux, together with a mitogen-activated protein kinase (MAPK) cascade will trigger immunity gene expression in the nucleus, in which, for example, WRKY transcription factors play important roles. MAP kinases are regulated by the ROS sensory kinase oxidative signal-inducible 1 (OXI1). At the same time, Ca^{2+} flux and phosphorylation by Botrytis-induced kinase 1 (BIK1), CDPKs, and calcineurin B-like protein (CBL)/CBL-interacting protein kinase (CIPK) modules can enhance the activity of plasma membrane localized respiratory burst oxidase homologs (RBOHs) D and/or F (RBOHD/F) to produce apoplastic ROS ($O_2^{\bullet-}/H_2O_2$). Peroxidases 33 and 34 (PRX33/34) contribute to apoplastic ROS generation for the oxidative burst. Within 20 min of pathogen perception, a Ca^{2+} flux is generated in the chloroplast, which is regulated by the thylakoid associated calcium-sensing protein (CAS). Pathogen perception might be signaled to the chloroplast by a MAPK cascade, direct transfer of calcium from the cytosol to the chloroplast or H_2O_2 coming from the oxidative burst (or a combination thereof). Downstream retrograde signaling to the nucleus might involve the ROS $^1O_2$ (mainly generated by photosystem II [PSII]) and $O_2^{\bullet-}$ (mainly generated by photosystem I [PSI]). Executer1 and 2 (EX1/2) act downstream of $^1O_2$ to alter nuclear gene expression. The central immune regulator enhanced disease susceptibility 1 (EDS1) has been implicated downstream of chloroplastic $O_2^{\bullet-}$ and interacts with phytoalexin deficient 4 (PAD4) and Senescence-Associated gene 101 (SAG101) as heterodimers to alter nuclear immunity gene expression. **(B)** A later response to pathogen infection involves the ROS sensory protein lesion simulating disease 1 (LSD1), which was postulated to inhibit spread of cell death lesions mediated by metacaspase 1 (MC1) during hypersensitive response (HR) type cell death (indicated by darkened color and membrane perforations). Enhanced immunity gene expression leads to increase of the immune hormone salicylic acid (SA) in the chloroplast (indicated by an upwards pointing arrow). A spreading SA signal, calcium fluxes, and ROS signal (putatively via RBOHD/CDPK5 relay) could signal to the ROS sensory protein *Arabidopsis* nonexpressor of pathogenesis-related genes 1 (NPR1) to activate systemic acquired resistance (SAR) in distal cells of the site of infection. Proteins connected to Ca^{2+} signaling are denoted in purple. Proteins connected to ROS signaling are shown in green. Hypothetical connections are broken. Abbreviations: CGNC, Ca^{2+}-permeable cyclic nucleotide-gated channel; ER, endoplasmic reticulum; iGluR, ionotropic glutamate receptor-like channels; PM, plasma membrane; ACA4, 8, 10, 11, 12, autoinhibited calcium-ATPase 4, 8, 10, 11, 12.