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Enhanced disease resistance to *Botrytis cinerea* in *myb46* Arabidopsis plants is associated to an early downregulation of *CesA* genes

Vicente Ramírez, Javier García-Andrade and Pablo Vera*

Instituto de Biología Molecular y Celular de Plantas (IBMCP); Consejo Superior de Investigaciones Científicas (CSIC); Universidad Politécnica de Valencia; Valencia, Spain

The cell wall is a protective barrier of paramount importance for the survival of plant cells. Monitoring the integrity of the cell wall allows plants to quickly activate defense pathways to minimize pathogen entry and reduce the spread of disease. Counterintuitively, however, pharmacological effects as well as genetic lesions that affect cellulose biosynthesis and content confer plants with enhanced resistance against necrotrophic fungi. These kind of pathogens target cellulose for degradation to facilitate penetration and to generate glucose units as a food source. Our results point towards the existence of a transcriptional reprogramming mechanism in genes encoding cellulose synthases (*CesAs*) that occurs very soon after *Botrytis cinerea* attack and that result in a temporary shut down of some *CesA* genes. Interestingly, the observed coordinated downregulation of *CesA* genes is more pronounced, and occurs earlier in *myb46* mutant plants. In the resistant *myb46* plants, pathogen infection induces transient downregulation of *CesA* genes that concur with a selective transcriptional reprogramming in a set of genes encoding structural cell wall proteins and extracellular remodeling enzymes. Together with previous indications, our results favor the hypothesis that CesAs are part of a surveillance system of the cell wall integrity that senses the presence of a pathogen and transduces that signal into a rapid transcriptional reprogramming of the affected cell.

Key words: necrotrophs, cellulose, cell wall, peroxidase, *B. cinerea*

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*Correspondence to: Pablo Vera;
Email: vera@ibmcp.upv.es

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The cell wall is a dynamic complex composite of cellulose, hemicellulose, pectin, lignin and proteins, among other constituents, that is constantly remodeled during growth and development and in response to environmental cues.[1] In addition to providing structural support, it controls cell expansion and is involved in the exchange of water and substances throughout plant development. It is also the first defensive structure that many pathogens encounter before confronting intracellular plant defenses, not only as a passive barrier but also constituting a reservoir of antimicrobial compounds and an important sensory component for downstream signaling pathways. In fact, it has been proposed that in plants, cell damage may be sensed by detection of modification of polysaccharides, release of oligosaccharides, inhibition of cell wall synthesis or assembly or deformation of the plasma membrane adjacent to damaged and weakened cell walls.[2-4] As cellulose is the most abundant polymer and the major load-bearing polysaccharide common to both primary and secondary cell wall, pathogens target it for degradation to facilitate penetration and to generate glucose units as a food source. How the plant cell detects a defect in cellulose synthesis and deposition is unknown, but it may be possible that plant cells have developed a surveillance mechanism to monitor the plasma membrane-localized cellulose synthase complex, the crystallinity and content of cellulose produced or even the generation and release of degraded cellulose fragments.[5] Such a system might be similar to the cell wall integrity-sensing system found in yeast cells that responds to perturbations in the cell wall structural integrity through the

action of stress sensors thought to act as cell surface mechanosensors.[6,7]

Cellulose consists of long parallel linear β-1,4-D-glucan chains that are assembled into crystalline microfibrils by hydrogen bonding by a large multimeric complex containing at least three different cellulose synthase (CesA) enzymes located at the plasma membrane.[8,9] Arabidopsis genome holds 10 *CesA* genes, six of which encode proteins with known functions.[1] So far, CesA1 (RADIAL SWELLING1 [RSW1]), CesA3 (ISOXABEN RESISTANT1 [IXR1]/ CONSTITUTIVE EXPRESSION OF VSP1 [CEV1]), CesA6 (PRC1/IXR2), CesA2, CesA5 and CesA9 have been associated with the CesA complexes active during primary wall formation, while CesA4 (IRREGULAR XYLEM5 [IRX5]), CesA7 (IRX3) and CesA8 (IRX1) have been reported to be part of the CesA complex responsible for secondary wall cellulose synthesis, which takes place after the arrest of cell expansion (reviewed in ref. 10).

Cell wall synthesis is a process highly regulated at the transcriptional level. A group of transcription factors in the NAC and MYB families seems to represent a core set of master regulators of secondary cell wall formation, including SND1,[11] MYB46,[12,13] NST1 and NST3,[14] and MYB58 and MYB63,[15] among others. We have shown[16] that the Arabidopsis transcription factor MYB46, previously described to regulate secondary cell wall biosynthesis in the vascular tissue of the stem, is pivotal for mediating disease susceptibility to the fungal pathogen *Botrytis cinerea*. Different *myb46* knock-down mutants exhibit increased disease resistance to *B. cinerea*, a phenotype that is accompanied by selective transcriptional reprogramming of a set of genes encoding cell wall proteins and enzymes, of which extracellular type III peroxidases are conspicuous. We hypothesized that defense-related signaling pathways and cell wall integrity are interconnected, and MYB46 likely functions as a disease susceptibility modulator to *B. cinerea* through the integration of cell wall remodeling and downstream activation of secondary lines of defense.[16]

In this work we further investigated the link between cell wall modifications and heightened resistance to *B. cinerea* in *myb46* plants, in comparison to wild type plants, by establishing a relationship between infection and a transcriptional regulatory network that controls expression of *CesA* genes. Although total cellulose accumulation appears uncompromised in *myb46* plants,[16] we wondered if at least some of the signaling caused by *B. cinerea*-induced cell wall disruption could be caused by modification of the cellulose network.

Previous studies reported a connection between cellulose and disease resistance to fungal pathogens. In this regard, inhibition of cellulose synthesis with the herbicide isoxaben induces jasmonic acid (JA) synthesis, a stress phytohormone that regulates response to necrotrophic pathogens as well as the concurrent activation of some defense-associated genes such as the defensine *PDF1.2*.[17-19] Similarly, mutations in *CesAs* for either the primary or secondary cell wall induce a number of stress and defense-like responses and also enhance disease resistance to pathogens. For example, cellulose deficiency in the primary cell wall mutant *cesa3* constitutively elicits ethylene (ET) and JA signaling and enhances disease resistance to fungi and aphids.[20,21] Also, disruption of the secondary cell wall (e.g., *cesa4*, *cesa7* and *cesa8* mutants) causes an increase of the disease resistance to the necrotrophic fungi *B. cinerea* and *Plectosphaerella cucumerina*.[22] However, we were not aware of any study reporting on the transcriptional modulation of *CesA* genes following fungal infection. Therefore, we measured transcript abundance for representative members of the *CesA* gene family by RT-qPCR during a 72-h time course following inoculation of Col-0 with *B. cinerea*. These studies were comparatively performed with *myb46-2* plants (**Fig. 1**). Our results demonstrated that for all *CesA* genes analyzed, there was a general downregulation in gene expression following fungal inoculation that become apparent at 24 h.p.i. Exclusive of *CesA3*, downregulation was temporary as transcript abundance recovered, and in some cases even exceeded normal levels at 72 h.p.i. The induced repression of *CesA3* remained even at 72 h.p.i. The effect on repression of the *CesA* gene was also reproduced in *myb46-2* plants, but the mutant plants demonstrated a reproducible tendency to heighten the degree of repression observed in Col-0. This was most evident in *CesA3* and *CesA4*, the two genes that become most rapidly and abruptly repressed following fungal infection. In addition, *CesA3* and *CesA4* transcripts were nearly undetectable in *myb46-2* plants at 24–48 h.p.i. *CesA8,* and to a lesser extend *CesA7*, were also notably repressed following inoculation with *B. cinerea*. *myb46-2* plants showed constitutive reduced transcript levels for *CesA8*. *CesA8* exhibited marked expression recovery at 72 h.p.i.; a recovery that was slowed in *myb46-2* plants. As a control in these RT-qPCR experiments we used the defensin *PDF1.2a* and the basic chitinase *PR-3* marker genes whose expression patterns were opposed to those of *CesA* genes and became highly activated during the infection process.

Several fungi use topographical cues on the plant surface to guide them towards a suitable entry point, and it may be that the cell wall composition could constitute one of these fingerprints that pathogens read to find the appropriate penetration point. Accordingly, alterations in the cellulose disposition, composition or proprieties (e.g., in *cesa* mutants) could change the topographic code and in turn impacting on the disease susceptibility. Our data, together with results indicating that genetic defects in cellulose biosynthesis of primary and secondary cell walls provide plants with increased disease resistance to fungal pathogens, favor the hypothesis that CesAs are part of a surveillance system of the cell wall integrity that senses the presence of a pathogen and transduce that signal into a rapid transcriptional reprogramming of the affected cell.[23,24] If MYB46 is functioning as a modulator of the cell wall assembly, it is plausible that *myb46* mutant plants become more sensitive to the presence of *B. cinerea* and in turn respond with a more efficient defense response that is primarily activated at the cell wall level. Recognition of the intruder at the cell wall sets in motion an early and very precise transcriptional reprogramming; for Arabidopsis, one of its own constituents, the *CESA* genes, that may help adjust the cell wall network

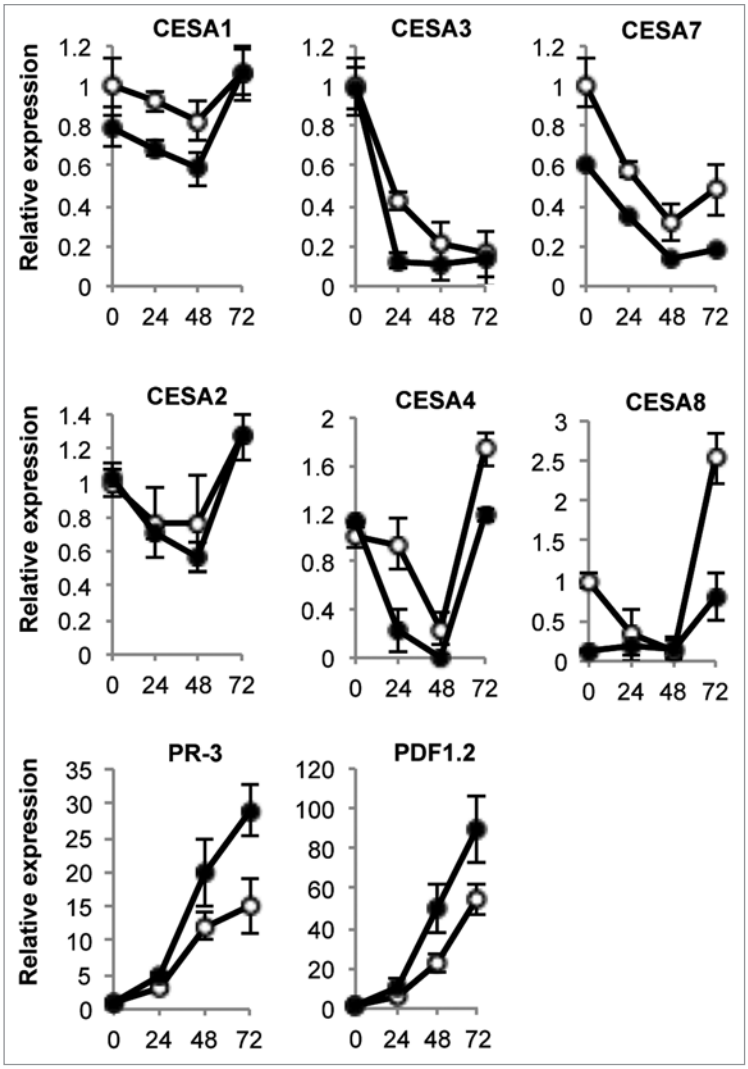

Figure 1. Expression of *CesA* genes in leaves at early stages of *B. cinerea* infection. Relative expression was assayed over a 72-h time course by RT-qPCR on total RNA from leaves of Col-0 (open circles) or *myb46-2* (filled circles) plants after inoculation with a *B. cinerea* spore suspension. Expression was normalized to *actine2* (*ACT2*) gene expression. For ease of comparison, an arbitrary relative expression value of 1 was assigned to each *CesA* gene and refers to the expression level attained in Col-0 plants at 0 h.p.i. with *B. cinerea*. Error bars represent standard deviation of three independent replicates.

to this new extracellular scenario. The existence of such a surveillance system for plant cell wall integrity following pathogen disruption has been previously proposed,[24,25] and seems to be a component of early signaling events that must subsequently be interconnected with known plant defense networks.

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