Plant Signaling & Behavior 7:2, 210–212; February 2012; © 2012 Landes Bioscience

Role of glutathione in plant signaling under biotic stress

Carole Dubreuil-Maurizi and Benoît Poinssot*

Université de Bourgogne; UMR Plante Microbe Environnement; INRA UMR 1088; CNRS UMR 5184; Dijon, France

Keywords: glutathione, *pad2-1* mutant, defence response, redox environment, *Arabidopsis thaliana*, *Phytophthora brassicae*

Glutathione (GSH) is a non-protein thiol compound which has been repeatedly reported to play an important role in plant responses during biotic stresses. However, our knowledge of glutathione-related molecular mechanisms underlying plant defense responses still remains limited. We first discovered that the *Arabidopsis thaliana phytoalexin deficient 2-1* (*pad2-1*) mutant was linked to glutathione deficiency since the mutation was identified in the *GSH1* gene encoding the first enzyme of glutathione biosynthesis: Glutamate Cysteine Ligase (GCL). Interestingly, this glutathione-deficient mutant *pad2-1* also displays a high susceptibility to a wide range of invaders. We recently reported that the glutathione deficiency in *pad2-1* is directly related to a low content of GCL protein. In parallel, we highlighted that the altered redox potential in *pad2-1* upregulates the oxidative-stress marker genes *GR1*, *GSTF6* and *RbohD* during infection with the hemibiotrophic oomycete *Phytophthora brassicae*. Moreover, the impairment of early signaling events such as plasma membrane depolarization, production of nitric oxide and reactive oxygen species also correlates with the reduced hypersensitive response (HR) observed during *P. brassicae* infection. Concerning the impaired salicylic acid (SA)-dependent pathway in *pad2-1*, our results indicated that transcripts of IsoChorismate Synthase1 (ICS1, a main enzyme of SA biosynthesis) do not accumulate in response to pathogen. In this review, we integrate previous knowledge and recent discoveries about *pad2-1* to better understand the involvement of glutathione in the *pad2-1* pleiotropic phenotype observed during biotic stresses.

Glutathione (γ-glutamylcysteinylglycine) is an abundant and ubiquitous low-molecular-weight thiol in all aerobic organisms. The presence of cysteine confers its biological properties mainly as antioxidant function through its involvement in cell redox homeostasis. It has been largely reported for more than 20 years to play an essential role in many cellular processes as development, growth or environmental response in plants.[1] Although the involvement of glutathione in plant responses to pathogens has been described, underlying molecular mechanisms still remain to be discovered. Using forward genetic approaches, we are now able to carry out thorough research on the role of glutathione in cell processes. Several mutants in *Arabidopsis thaliana* have been identified to contain low glutathione amounts compared with wild type and are related to mutations in the *GSH1* gene (At4g23100) encoding Glutamate Cysteine Ligase (GCL), the first enzyme of glutathione biosynthesis. These glutathione-deficient mutants display a large range of phenotypes such as altered development, enhanced abiotic stress sensitivity as well as pathogen susceptibility: - *rml1* (*root-meristemless1*; ~3% of wild-type GSH; abortion in plant development[2]); - *rax1-1 (regulator of APX2 1-1*; ~40% of wild-type GSH; sensitivity to high light[3]); - *zir1* (*zinc tolerance induced by iron 1*; ~15% of wild-type GSH; deficiency in Fe-mediated Zn tolerance[4]); - *cad2-1 (cadmium-sensitive 2-1*; ~30% of wild-type GSH; sensitivity to heavy metals and moderate susceptibility to pathogens[5,6]); and -*pad2-1 (phytoalexin-deficient 2-1*; ~20% of wild-type GSH; susceptibility to pathogens[6]; and deficiency in Fe-mediated Zn tolerance[4]). Of these, the *pad2-1* mutant was defined as the best candidate to highlight the key role of glutathione in signaling processes leading to plant resistance to pathogens. Indeed, the low content of glutathione in *pad2-1* confers an enhanced susceptibility to various fungal, bacterial and oomycete pathogens (*Botrytis cinerea, Alternaria brassicicola, Pseudomonas syringae, Phytophthora brassicae*) as well as insect herbivores (*Spodoptera littoralis*).[6-15] Supporting this phenotype in *pad2-1*, many studies reported deficiencies in defense responses as (1) the low accumulation of antimicrobial defense compounds camalexin and indole glucosinolates, and (2) the altered salicylic acid (SA)-dependent pathway with a low accumulation of SA correlated with an impaired *PR-1* gene expression. All these responses are altered in *pad2-1* under *P. brassicae* infection and lead to plant hypersusceptibility.[10,15]By using *P. brassicae* pathogen or oligogalacturonide elicitor, we showed that glutathione deficiency of *pad2-1* affects most cellular events including oxidative stress-related events, early signaling events, defense gene expression and hypersensitive response (HR). Here, we present these new data which improve the understanding of the role of glutathione in plant defense mechanisms. We propose a summary figure to establish the link between the glutathione deficiency and the susceptibility of *pad2-1* to pathogens, especially *P. brassicae* (**Fig. 1**).

The Deficiency of Glutathione is Related to a Low Content of GCL Protein

Since *pad2-1* displays a normal *GCL* transcript accumulation compared with wild type, we studied GCL at protein level to

*Correspondence to: Benoît Poinssot; Email: bpoinssot@dijon.inra.fr
Submitted: 11/21/11; Accepted: 11/22/11
http://dx.doi.org/10.4161/psb.18831

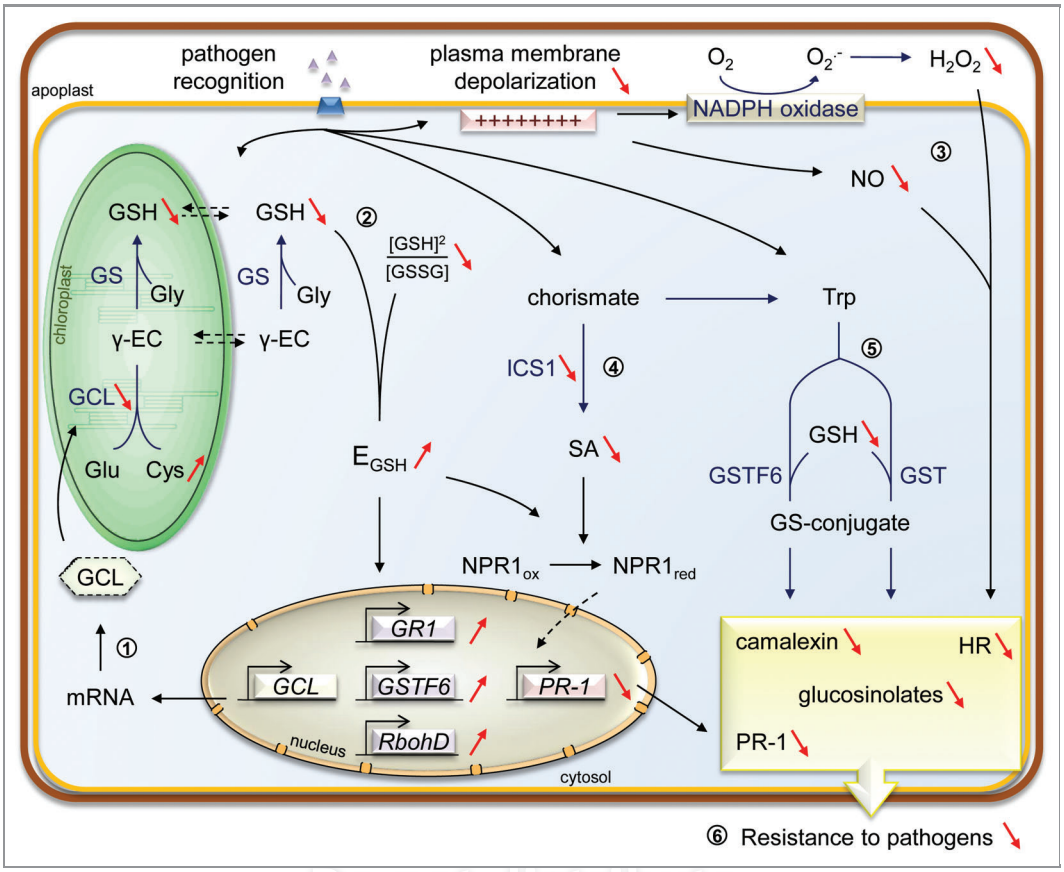

Figure 1. Overview of the glutathione depletion impact on defense signaling processes in *pad2-1* mutant under biotic stress. Modification of corresponding molecules or events in *pad2-1* mutant compared with wild type are represented with red arrows; γ-EC, γ-glutamylcysteine; Cys, cysteine; E_{GSH}, glutathione redox potential; GCL, glutamate cysteine ligase; Glu, glutamate; Gly, glycine; GR1, glutathione reductase1; GS, glutathione synthase; GS-conjugate, glutathione-conjugated compound; GSH, reduced glutathione; GSSG, oxidized glutathione; GST(F6), glutathione-S-transferase(F6); HR, hypersensitive response; ICS1, isochorismate synthase1; NO, nitric oxide; NPR1ox/red, oxidized/reduced non-expressor of pathogenesis-related genes1; PR-1, pathogenesis-related 1; RbohD, Respiratory burst oxidase homolog D; SA, salicylic acid; Trp, tryptophan.

determine the cause of the low content of glutathione. Structural prediction of GCL revealed that the *pad2-1* mutation (S298N) does not affect the ternary structure of GCL and probably not its enzymatic activity contrary to *cad2-1* and *rax1-1* mutations localized in substrate binding sites. However, we showed that *pad2-1* contains only 48% of wild-type GCL amount.[16] Based on these results, one hypothesis suggests that *pad2-1* mutation could affect GCL protein folding or degradation leading to glutathione deficiency and correlated with cysteine accumulation (**Fig. 1.1**).

Pad2-1 Senses a Permanent Oxidative Stress

From many years, glutathione has been shown to be a key molecule of the cellular redox homeostasis through its oxidized/reduced ratio as well as its total concentration. Using a cytosolic targeted redox-sensitive fluorescent probe GRX1-roGFP2, we observed in vivo that glutathione redox potential (E_{GSH}) is less reducing in *pad2-1* than in wild type under normal conditions (**Fig. 1.2**). This result was confirmed with the predominant oxidized form of GCL in *pad2-1* although the reduced form is observed in wild type.[16] Thus, based on many studies about regulation of gene expression by the redox state, we assume that this altered redox environment could influence gene expression under biotic stress. In that way, marker genes of oxidative stress such as *GR1* (*Glutathione Reductase1*), *GSTF6* (*Glutathione-S-Transferase F6*) and *RbohD* (*Respiratory Burst Oxidase Homolog D*) are upregulated in *pad2-1* mutant compared with wild type during *P. brassicae* infection.[16]

Glutathione Deficiency in *pad2-1* Affects Defense-related Signaling Events, which Confers a Susceptibility to Pathogens

In response to biotic stress, *pad2–1* displays altered early signaling events such as plasma membrane depolarization and production of H_2O_2 and nitric oxide (NO; **Figure 1.3**).[16] Our hypothesis is that the less reducing E_{GSH} could affect the corresponding gene expression and/or enzyme activity or upstream signaling events. From these results and based on previous studies, their impairment might explain the weak HR establishment observed in *pad2-1* during *P. brassicae* interaction.[16] In parallel, the SA-dependent pathway is blocked in *pad2-1* in response to

P. brassicae (**Fig. 1.4**).[10] Based on our results, we suggest that the abolished expression of *IsoChorismate Synthase 1* (*ICS1*), encoding a main enzyme of SA biosynthesis, is the origin of the low SA accumulation during *P. brassicae* infection.[16] Consequently, the expression of defense gene *PR-1* is not upregulated in *pad2-1*. Moreover we propose that the low content of glutathione directly affects the accumulation of the antimicrobial compounds camalexin and glucosinolates in *pad2-1* since glutathione has been reported to be a sulfur source in their biosynthesis (**Fig. 1.5**).[17,18] Finally, these low defense responses confer a susceptibility of *pad2-1* to pathogens (**Fig. 1.6**). In conclusion, we provided strong evidence that glutathione regulates early signaling events, stress-related gene expression and plant defenses by studying *pad2-1* mutant under biotic stress conditions.

Acknowledgments

C.D.-M. was supported by a grant from the French Ministère de l'Enseignement Supérieur et de la Recherche. This work was financially supported by the "Conseil Régional de Bourgogne."

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