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# Molecular Plant

## Redox and acetylation coordinate ABA-independent osmotic stress response --Manuscript Draft--

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<b>Opposed Reviewers:</b>	

Dear Editor of Molecular Plant,

Thank you for evaluating our manuscript. We have now corrected the manuscript according to the reviewer comments. Modifications to the figure and figure legend have been made. Also, a reference has been changed to consider S-nitrosylation of SnRK2 (lines 60-62). Those modifications are highlighted in the word document.

We hope that the new version of our manuscript will be convenient for publication.

Sincerely,

Jean-Philippe Reichheld

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## Redox and acetylation coordinate ABA-independent osmotic stress response

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15 **Running title:**

GPX1-mediated oxidation of bZIP68 transcription factor

## MAIN TEXT

Osmotic stress caused by drought or high salt concentrations is a major threat that limit plant development and affect crop productivity. In the context of climate change, understanding the mechanisms by which plant perceives their environment to adapt its development is a major question. A common feature of plants being exposed to osmotic stress is the accumulation of excessive reactive oxygen species (ROS) (Martinière et al., 2019). ROS can oxidatively damage biological molecules like nucleic acids, proteins and lipids, but also play important signaling functions. To protect from ROS-induced oxidation, living organisms have developed a large panel of enzymatic and non-enzymatic systems to remove ROS and oxidized molecules. Non-enzymatic antioxidants are represented by carotenoids, tocopherols, reduced glutathione (GSH) and ascorbate, as the enzymatic systems rely on superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX), peroxiredoxins (PRX) and glutathione peroxidases (GPX). While most of these molecules have been largely documented, relatively little is known about GPXs functions, particularly in plants (Passaia et al., 2014). Biochemically, GPXs catalyse the reduction of different types of oxidized molecules like H<sub>2</sub>O<sub>2</sub>, organic and lipid peroxides. While mammals GRX harbour a selenocysteine-containing catalytic site and are generally using glutathione (GSH) as reducing power, plant GPX have a disulfide catalytic site which are using thioredoxin (TRX) as reducing system (Iqbal et al., 2006). Beside their peroxide detoxification functions, GPXs may also act as a signaling molecule through its capacity to be modified by H<sub>2</sub>O<sub>2</sub> and to transfer this redox signal to other molecules. The seminal evidence for this role came from the GPX3 (ORP1) isoform in *Saccharomyces cerevisiae*. ORP1 is primarily oxidized by H<sub>2</sub>O<sub>2</sub> on the peroxidatic Cys residue of its catalytic site, forming a sulfenic acid residue. This highly redox-reactive residue then forms a mixed disulfide with a Cys in the transcription factor YAP1, a key factor of the transcriptional response to oxidative stress in yeast. Subsequently, a nucleotidic attack of this mixed disulfide by a second release ORP1, while forming an intramolecular disulfide bond in YAP1, leading to its activation

through a conformational changes of its NES sequence and hence its accumulation in the nucleus (Delaunay et al., 2002). In plants, the Arabidopsis GPX3 isoform has been involved in stress response H<sub>2</sub>O<sub>2</sub>-signaling. Miao et al. (Miao et al., 2006) showed higher drought stress sensitivity of a *gpx3* KO mutant, associated to higher sensitivity to H<sub>2</sub>O<sub>2</sub> and enhanced H<sub>2</sub>O<sub>2</sub> production in guard cells. In addition to scavenge H<sub>2</sub>O<sub>2</sub>, GPX3 is proposed to play an important role in ABA-mediated drought stress response by the oxidative-dependent inactivation of 2C-type protein phosphatase ABI2 (and ABI1 to a lesser extent), key repressor of ABA signaling (Miao et al., 2006). As a result, stomatal closure and ABA-response gene expression was impaired in the *gpx3* mutant. In rice, the mitochondrial isoform OsGPX3 has also been involved in the ABA response. Rice GPX3 knock-down plants show altered ABA responses, such as germination, ROS accumulation, stomatal closure and dark-induced senescence (Paiva et al., 2021). Another presumed mitochondrial isoform, OsGPX1, was also involved in the response to salinity in rice plants.

In this report, Zhou et al. (Zhou et al., 2021) point to the critical role of the thiol peroxidase GPX1 in osmotic stress response in rice. Interestingly, this role is independent on ABA response pathway, but involves a redox regulation of bZIP68, a VRE-like basic leucine zipper (bZIP) transcription factor implicated in ABA-independent osmotic stress response pathway. This emphasizes the key roles of GPX and more generally redox regulation in modulating plant responses to drought and osmotic stresses, by controlling both ABA-dependent and independent pathways (Figure 1). Upon drought stress, SnRK2 protein kinases, core components of ABA signaling which interact with PP2C, are regulated by the coordination of phosphorylation and redox (S-persulfidation, [S-nitrosylation](#)) regulation of their activity (~~Zhang et al., 2021~~[Zhang et al., 2021](#)).

Zhou et al. showed that, upon exposure to osmotic stress, GPX1 is quickly oxidized and forms an intramolecular disulfide bond. Similarly to the yeast GPX3-dependent YAP1 activation, a disulfide exchange occurs between GPX1 and bZIP68, leading to the activation of bZIP68. However, while in yeast, GPX3 activates YAP1 by triggering its nuclear accumulation, rice GPX1 induces bZIP68 homo-

tetramerization and direct binding to the AGCTG (VRE-like) motif within the promoters of osmotic-stress response genes. This regulation also differs from bZIP factors binding to other types of cis-elements, which undergo oligomerization in response to H<sub>2</sub>O<sub>2</sub>, and leading to inhibition of their DNA binding activity (Shaikhali et al., 2012). Rather, GPX1-dependent bZIP68 homo-tetramerization is reminiscent of the H<sub>2</sub>O<sub>2</sub>-dependent activation of the OxyR transcription factor in *E.coli*, which DNA binding is activated through disulfide bond homo-tetramerization (D'Autréaux and Toledano, 2007).

Zhou et al. also discovered that the nuclear translocation of GPX1 is not dependent on its redox state but is rather regulated by acetylation. Indeed, treatments with general inhibitors of histone, or rather lysine, deacetylases led to marked nuclear accumulation of GPX1, which was not observed under H<sub>2</sub>O<sub>2</sub> treatment. Protein acetylation generally occurs on lysine residues of proteins and are deposited or removed by lysine acetyltransferases (KAT) or lysine deacetylases (KDAC), respectively. As it modify lysine positive charge, acetylation can affect many biological functions of target proteins, like enzymatic activity, protein conformation or cellular localization. How acetylation is affecting GPX1 nuclear translocation is a key question. Depending on the acetylated residues, it might change GPX1 conformation, mask nuclear translocation domains or interactions with docking proteins. The identification of the KAT and KDAC specifically involved in GPX1 regulation under osmotic stress would also help to uncover other partners of the pathway.

The observation of the cytosolic/nuclear localization of GPX1 contrasts with previous reports suggesting a mitochondrial localization of the rice GPX1, which is consistent with an N-terminal extension in OsGPX1. Many examples exist of proteins located in mitochondria, cytosol and nucleus, and in most cases, subcellular localization is driven by alternative transcription, translation or splicing signals. The presence of two in-frame translation initiation codons might explain an alternative mitochondrial/cytosolic localization of OsGPX1 in the context of osmotic stress conditions.

Another point of interest would be to investigate if/how the GPX1 redox-regulation of bZIP68 interferes with the peroxidatic activity of GPX1. By the way, it is interesting to notice that the regulatory disulfide bond in GPX1 does not involve the catalytic cysteine. It would also be interesting to know how oxidized bZIP68 homo-tetramers are reduced and to identify actors implicated in osmotic stress recovery mechanisms. Thiol reductases like thioredoxins are good candidates to participate to this pathway.

In conclusion, the work by Zhou et al. provides a new molecular mechanism involving a crosstalk between acetylation and redox to regulate osmotic stress response. How these two types of posttranslational modifications are coordinated to fine-tune the pathway will require further exploration, but the fact that similar regulation mechanisms are conserved in different living kingdoms suggests that they are key for adaptation of living organisms to environmental constraints.

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## 110 **AUTHOR CONTRIBUTION**

CB and JPR wrote the article and designed the figure.

## **CONFLICT OF INTEREST**

We declare no conflict of interest.

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## FIGURE LEGENDS

### Figure 1. GPX are core components of plant responses to drought and osmotic stress.

Zhou et al demonstrate that GPX1 is oxidized in response to ROS, in an ABA-independent way, which results in an internal disulfide bond. This disulfide bond is reduced by two sulfenylated cysteines from bZip68, thus leading to the homotetramerization of this bZIP, and the activation of osmotic stress-responsive genes such as *DREB1* or *COR413-TM1*. It is still unclear in which compartment this oxidation takes place, GPX1 moving from the cytosol to the nucleus upon acetylation (Ac). Works in rice and Arabidopsis also suggest an important role for GPX3 in drought and osmotic stress responses, notably by participating in the ROS-dependent positive feedback loop by inactivating PP2C. Indeed, in Arabidopsis, oxidized PP2C are unable to inactivate SnRK2 that phosphorylate targets such as NADPH oxidases (RBOH) and transcription factors controlling downstream genes including *GPX3* itself. ABF and bZIP transcription factors finally regulate the expression of drought and osmotic stress responsive genes, part of them being common targets of ABA-dependent and -independent pathways. The subscripts red and ox indicate the reduced and oxidized forms of the protein, respectively.

