Environmental and Experimental Botany 150 (2018) 127–140

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Comprehensive analysis of antioxidant mechanisms in *Arabidopsis* glutathione peroxidase-like mutants under salt- and osmotic stress reveals organ-specific significance of the AtGPXL's activities

Krisztina Bela[a], Riyazuddin Riyazuddin[a], Edit Horváth[a,b], Ágnes Hurton[a], Ágnes Gallé[a], Zoltán Takács[a], Laura Zsigmond[b], László Szabados[b], Irma Tari[a], Jolán Csiszár[a,*]

[a] Department of Plant Biology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52., H-6726 Szeged, Hungary
[b] Institute of Plant Biology, Biological Research Centre of HAS, Temesvári krt. 62., H-6726 Szeged, Hungary

ARTICLE INFO

Keywords:
Antioxidative mechanisms
Arabidopsis thaliana
Ascorbate-glutathione pool
Glutathione peroxidase activity (GPOX)
Thioredoxin peroxidase activity (TPOX)

ABSTRACT

Plant glutathione peroxidases contain cysteine in their active site instead of selenocysteine, and most of them use the thioredoxin (TRX) system more efficiently than the glutathione (GSH) system during the reduction of H_2O_2 and lipid peroxides. Recently, the more precise glutathione peroxidase-like (GPXL) name was adopted for the eight *Arabidopsis thaliana* isoenzymes. In this paper we have compared the effect of osmotic and salt stresses on the 6-week-old T-DNA insertion mutants (*Atgpxl1-8*) grown hydroponically. The glutathione peroxidase (GPOX) activity measured with cumene hydroperoxide substrate in the wild type *Arabidopsis* shoots and roots was 2–5 times higher than the thioredoxin peroxidase (TPOX) activity. Mutation in one of the eight *AtGPXLs* resulted in decreased TPOX activity in untreated shoots and, in contrary to the wild type, this activity did not increase under stress, verifying the connection with TRX system. The level of reduced ascorbate significantly altered in shoots and the amount of GSH in roots under both control conditions and after 2 days of stress treatments. While positive correlations were found between GSH and TPOX activity in the wild type shoots and roots, the connection between the AtGPXLs and the GSH pool was stronger in roots than in shoots. Nevertheless, the TPOX activity increased in *Atgpxls* roots when the GSH content decreased, indicating the relationship between the GSH and TRX systems. The *AtGPXL* expression in mutant plants showed that some isoenzymes are regulated jointly. Furthermore, while *AtGPXLs* were generally down-regulated, several stress-inducible transcription factor genes were up-regulated, especially after applying osmotic stress.

1. Introduction

In plant cells, disadvantageous environmental conditions increase the production of reactive oxygen species (ROS). The ROS, such as superoxide radical, hydroxyl radical or hydrogen peroxide (H_2O_2), can induce detrimental oxidation of macromolecules including lipids, proteins and nucleic acids. In order to minimize ROS-derived damages and to keep its levels tightly regulated, a series of non-enzymatic antioxidants including but not limited to ascorbate (ASC), glutathione (GSH), carotenoids and tocopherols, as well as a set of enzymatic ROS detoxification systems have evolved in aerobic organisms which are represented in various combinations in each intracellular organelle (Noctor et al., 2014). Among the enzymes, the catalase (CAT) and different peroxidases may be implied in mass scavenging of H_2O_2 (Asada, 1992; Willekens et al., 1995). Guaiacol peroxidases (POXs) catalyse the reduction of H_2O_2 using electrons from various donor molecules (Passardi et al., 2004), the ascorbate peroxidase (APX) reduces H_2O_2 at the expense of ASC (Asada, 1988), while glutathione peroxidases (GPXs), glutathione transferases (GSTs), and peroxiredoxins (PRXs) reduce H_2O_2 and hydroperoxides by thiol-mediated pathways (Dietz et al., 2002; Chang et al., 2009). The role and mechanism of most of these enzymes in stress responses have been investigated intensively for several decades, even though relatively little is known about plant GPXs. While the evidence of their activity in different cultured plant cells was first reported already in 1985 (Drotar et al., 1985), several plant GST isoenzymes also have glutathione-

Abbreviations: ASC, ascorbate; APX, ascorbate peroxidase; CAT, catalase; CDNB, 1 chloro-2,4-dinitrobenzene; CHP, cumene hydroperoxide; DHA, dehydroascorbate; GPOX, glutathione peroxidase; GPXL, glutathione peroxidase-like; GR, glutathione reductase; GSH, reduced glutathione; GSSG, glutathione disulfide, oxidized glutathione; GST, glutathione transferase; MDA, malondialdehyde; POX, guaiacol peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TPOX, thioredoxin peroxidase; TRX, thioredoxin

* Corresponding author.
E-mail address: csiszar@bio.u-szeged.hu (J. Csiszár).

https://doi.org/10.1016/j.envexpbot.2018.02.016
Received 30 October 2017; Received in revised form 23 January 2018; Accepted 28 February 2018
Available online 12 March 2018
0098-8472/

dependent peroxidase activity (Roxas et al., 1997; Kilili et al., 2004; Basantani and Srivastava, 2007; Dixon and Edwards, 2009; Dixon et al., 2009), encumbering to clarify the significance of the particular GPX or GST enzymes.

The GPX (EC1.11.1.9) enzyme family comprises proteins phylogenetically related to non-heme thiol peroxidases that catalyse the reduction of H_2O_2 or organic hydroperoxides to water or the respective alcohols using reduced glutathione. Characterisation of numerous GPXs in various organisms revealed their broad substrate specificities and high affinity to H_2O_2 (Brigelius-Flohé and Flohe, 2003). Their ROS-scavenging role was proved in several reports (Arthur, 2001; Battin and Brumaghim, 2009; Yang et al., 2015). The mammalian GPXs are central components of the antioxidant metabolism and participate largely in the repair of biomembranes (McCay et al., 1976; Imai and Nakagawa, 2003; Islam et al., 2015). The plant glutathione peroxidase genes are closely related to animal phospholipid hydroperoxide glutathione peroxidases (Margis et al., 2008), however the isoenzymes contain cysteine instead of selenocysteine in their active site contrary to most of the animal GPXs (Herbette et al., 2007). While some of them have both glutathione peroxidase and thioredoxin peroxidase (TPX) functions, they prefer the thioredoxin (TRX) regenerating system *in vitro* rather than the glutathione system and even are regarded to be actually thioredoxin peroxidases (Herbette et al., 2002; Iqbal et al., 2006; Navrot et al., 2006; Lubos et al., 2011). They reduce more efficiently peroxides different from H_2O_2 such as organic hydroperoxides and lipid peroxides (Milla et al., 2003). Recently, Attacha et al. (2017) adopted the GPX-like (GPXL) nomenclature for the *Arabidopsis thaliana* isoforms to avoid any confusion resulting from protein names (http://www.arabidopsis.org/servlets/TairObject?type=gene_class_symbol&id=6531241478).

Passaia et al. (2014b) investigated the role of *Arabidopsis thaliana* GPXLs in shoot and root development using T-DNA insertion mutant lines. They found, that the shoot phenotypes were largely similar in *Atgpxl* mutants and wild type, however all mutants showed altered root phenotypes. They have confirmed the connections between the AtGPXLs and auxin, abscisic acid, strigolactone hormones, thereby demonstrating the importance of AtGPXLs in the hormone-mediated regulation of lateral root development. Earlier it was reported that redox processes involving glutathione and thioredoxins exert a strong influence on root architecture (Benitez-Alfonso et al., 2009; Bashandy et al., 2010), moreover GSH is involved in the interplay between auxin and strigolactone signaling that controls this process (Marquez-Garcia et al., 2014). It was suggested that GPXLs may be required to GSH- and reduced thioredoxin-mediated redox control of lateral root development (Passaia et al., 2014b).

The key redox pairs in the soluble phase of the cells are the NAD(P)H/NAD(P)$^+$, ascorbate/dehydroascorbate (ASC/DHA), glutathione/glutathione disulfide (GSH/GSSG), and reduced thioredoxin/oxidized thioredoxin (TRX_{red}/TRX_{ox}) – all of which are linked to the production or enhanced availability of ROS. There is a close interplay among the individual redox active molecules, and the status of each of them can influence the plant metabolism and environmental responses. The appropriate cellular response requires the presence of redox-sensitive proteins that can undergo reversible oxidation/reduction and may switch 'on' and 'off' depending upon the cellular redox state (Potters et al., 2010). Redox-sensitive metabolic enzymes may directly modulate corresponding cellular metabolism, whereas redox-sensitive signaling proteins execute their function via downstream signaling components, such as kinases, phosphatases, and transcription factors. A tight control is necessary to balance these activities and maintain coordination, including reversible redox regulation of proteins by dithiol-disulfide exchange, regulation of phosphoproteins, activation of signaling pathways by ROS-responsive regulatory genes (Mittler et al., 2004; Foyer and Noctor, 2005, 2012, 2013). According to present conception, ROS-producing enzymes, antioxidants and their redox states all contribute to the general redox homeostasis in the plant cell (Potters et al., 2010), but glutathione has been considered the master regulator of intracellular redox homeostasis (Foyer and Noctor, 2012; Noctor et al., 2012; Foyer and Noctor, 2013; Gill et al., 2013).

GSH (γ-Glu-Cys-Gly) is an abundant multifunctional tripeptide, which cooperates tightly with ASC in the Foyer–Halliwell–Asada cycle (Foyer and Noctor, 2012, 2013). Besides participating in the reduction of DHA, GSH also plays a key role in the direct ROS scavenging and in the protection of the thiol groups of proteins (Zagorchev et al., 2013). When GSH reacts with oxidants it becomes converted into glutathione disulfide. As a result of the reversible convertibility between the reduced and oxidized forms and the relatively high concentration of the GSH in the cells, glutathione is one of the most important redox buffer systems. It is indicated that beside the amount of the total glutathione, the ratio of the GSH and the GSSG may be an effective marker of cellular redox homeostasis. From the concentrations of reduced and oxidized glutathione the half-cell reduction potential ($E_{GSSG/2GSH}$) can be calculated (Schafer and Buettner, 2001). It was indicated that redox changes (e.g. alteration in H_2O_2 level, ascorbate and GSH concentrations or the ratio of their reduced/oxidized form), and the half-cell reduction potential of the GSH/GSSG couple were correlated with the level of stress tolerance (Soltész et al., 2011).

The involvement of several plant GPXLs in stress responses was already indicated (Bela et al., 2017). Analysis of their gene expression showed that GPXL mRNA steady-state levels usually increase under different biotic and abiotic stresses (Milla et al., 2003; Navrot et al., 2006; Diao et al., 2014; Gao et al., 2014). More detailed investigations revealed that defects of *AtGPXL3* reduced the drought stress tolerance: mutants displayed impaired stomatal closure, faster water loss and lower temperatures of leaves (Miao et al., 2006). Knock out mutation of *AtGPXL8* led to increased sensitivity to salt and osmotic stress compared to wild type (Gaber, 2011), and paraquat treatment suppressed the root growth more and increased the level of oxidized proteins in *Atgpxl8* plants (Gaber et al., 2012). The *Oryza sativa* OsGPXL1 mitochondrial enzyme was proved to be important for root growth, water use efficiency, both phases of photosynthesis and photorespiration under salinity (Lima-Melo et al., 2016). Also, depletion of OsGPXL5 negatively affected the tolerance of salt stress (Wang et al., 2017). Overexpression of wheat *GPXL* genes in Arabidopsis enhanced early tolerance to high salt stress; the transgenic plants showed higher germination rate and decreased growth inhibition by NaCl treatment (Zhai et al., 2013). It was proved that AtGPXL3 does not only have a scavenging function, but can also interact with 2C-type protein phosphatase abscisic acid (ABA) INSENSITIVE2, therefore it acts as an oxidative signal transducer in ABA and drought stress signaling (Miao et al., 2006). Characterisation of the Arabidopsis *root meristemless1 (rml1)* mutant, which has a defect in GSH biosynthesis, revealed altered expression of thioredoxin related genes, among others the *GPXL6*. Presumably that in case of severe glutathione deficiency GPXLs are inclined to use TRX as electron donor compared to GSH, in this way they may have a possible link function between the GSH- and TRX systems (Schnaubelt et al., 2015).

In this paper we have compared the effect of osmotic and salt stresses on hydroponically grown Arabidopsis *gpxl* mutants to investigate roles of individual isoenzymes in the ROS elimination and antioxidant responses. Our aim was to evaluate the connection between the redox states of the main antioxidant redox pairs (ASC and GSH) and the related antioxidant enzyme activities in shoot and root of the different *Atgpxl* mutants under control and stress conditions. Besides investigating the changes of the redox potentials we detected the transcription of *AtGPXLs* and several abiotic stress-related transcription factors (TFs) to reveal their relationship in stress response and in the maintenance of cell homeostasis.