The effects of redox controls mediated by glutathione peroxidases on root architecture in Arabidopsis thaliana

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Abstract

Glutathione peroxidases (GPXs) fulfill important functions in oxidative signalling and protect against the adverse effects of excessive oxidation. However, there has been no systematic characterization of the functions of the different GPX isoforms in plants. The roles of the different members of the Arabidopsis thaliana GPX gene (AtGPX) family were therefore investigated using gpx1, gpx2, gpx3, gpx4, gpx6, gpx7, and gpx8 T-DNA insertion mutant lines. The shoot phenotypes were largely similar in all genotypes, with small differences from the wild type observed only in the gpx2, gpx3, gpx7, and gpx8 mutants. In contrast, all the mutants showed altered root phenotypes compared with the wild type. The gpx1, gpx4, gpx6, gpx7, and gpx8 mutants had a significantly greater lateral root density (LRD) than the wild type. Conversely, the gpx2 and gpx3 mutants had significantly lower LRD values than the wild type. Auxin increased the LRD in all genotypes, but the effect of auxin was significantly greater in the gpx1, gpx4, and gpx7 mutants than in the wild type. The application of auxin increased GPX4 and GPX7 transcripts, but not GPX1 mRNAs in the roots of wild-type plants. The synthetic strigolactone GR24 and abscisic acid (ABA) decreased LRD to a similar extent in all genotypes, except gpx6, which showed increased sensitivity to ABA. These data not only demonstrate the importance of redox controls mediated by AtGPXs in the control of root architecture but they also show that the plastid-localized GPX1 and GPX7 isoforms are required for the hormone-mediated control of lateral root development.

Key words: Abscisic acid, auxin, glutathione, oxidative stress, redox regulation, root architecture, strigolactones.

Introduction

Plant cells contain a large array of antioxidant enzymes that control the metabolism of oxidants such as reactive oxygen species (ROS) and also play a role in redox signalling (Foyer and Noctor, 2009, 2011). The induction of antioxidant enzymes is a particularly important component of plant stress responses that limit oxidant-induced programmed cell death (PCD) (Wu et al., 2010). Within the antioxidant network of plant cells, ascorbate peroxidases reduce H₂O₂ at the expense of ascorbate, which is then regenerated by monodehydroascorbate and dehydroascorbate reductases (MDHAR and DHAR). In parallel, glutathione peroxidases (GPXs), glutathione S-transferases (GSTs), and peroxiredoxins (PRXs) reduce H₂O₂ and hydroperoxides by ascorbate-independent thiol-mediated pathways (Dietz et al., 2002; Chang et al. 2009). While many classes of GSTs display peroxidase activity (Dixon et al., 2009), in general they are only able to metabolize H₂O₂ at low rates (Mannervik, 1985).

GPXs are non-haem thiol hydroperoxidases, which catalyse the reduction of H₂O₂ or organic hydroperoxides to water or corresponding alcohols (Herbette et al., 2007). The active site...
of these enzymes is composed of a catalytic triad formed by selenocysteine/cysteine, glutamine, and tryptophan (Herbette et al., 2007). In contrast to the animal GPXs, which are selenium proteins containing a selenocysteine at the catalytic site, the plant enzymes do not bind selenium (Eshdat et al., 1997; Herbette et al., 2007; Toppo et al., 2008). Moreover, the animal GPXs exclusively use GSH as the reducing substrate but, despite their current nomenclature, the plant GPXs prefer reduced thioredoxin (TRX) as reductant and they have comparatively low activities with GSH (Iqbal et al., 2006; Navrot et al., 2006; Herbette et al., 2007). They are therefore better described as thiol peroxidases (Navrot et al., 2006).

GPXs are considered to play important roles in preventing oxidant-induced PCD. In Chlamydomonas reinhardtii, for example, a GLUTATHIONE PEROXIDASE HOMOLOGOUS (GPXH) gene is highly expressed in cells exposed to high light or singlet oxygen generation in the chloroplasts (Leisinger et al., 2001; Fischer et al., 2007; Ledford et al., 2007). Constitutive over-expression of GPXH together with GSTSI enhanced tolerance to singlet oxygen, suggesting that GPXH is important in defence against photooxidative stress.

GPXs interact with other proteins and hence they are considered to have signalling functions (Delaunay et al., 2002; Miao et al., 2006). Some members of the GPX superfamily can form tetrameric structures with other proteins in response to oxidants such as H2O2 or hydroperoxides. In yeast, for example, AtGPX8 protects the nucleus and cytosol from mitochondria (Margis et al., 2003). AtGPX8 interacts with protein phosphatase type 2C (PP2C) and with a transcription factor via an oxidation-induced event in order to promote the activation of genes encoding thiol-reducing and antioxidant proteins (Delaunay et al., 2002). The Arabidopsis AtGPX3 interacts with protein phosphatase type 2C (PP2C) proteins such as ABSCISIC ACID INSENSITIVE (ABI) 1 and ABI2, in order to activate plasma membrane Ca2+ and K+ channels that facilitate stomatal closure (Miao et al., 2006). Rice plants lacking GPX3 had shorter roots and smaller shoots than wild-type plants, indicating that GPX3 plays an essential role in root and shoot development (Passaia et al., 2013). Two stromal GPXs (AtGPX1 and AtGPX7) were shown to be important in chloroplast functions, particularly light acclimation and also in plant immune responses (Chang et al., 2009).

In Arabidopsis thaliana, GPXs are encoded by a small gene family, named AtGPX1 to AtGPX8. The enzymes products of these genes are considered to be important redox sensors as well as antioxidants, but the precise functions of the different GPX isoforms remains poorly characterized. Each GPX member localizes to a distinct cellular location. While there is some controversy in the literature regarding the intracellular localizations of AtGPX3 and AtGPX6, there is a consensus that AtGPX1 and AtGPX7 are localized to the chloroplasts, AtGPX4 and AtGPX5 are cytosolic, AtGPX2 is localized to the endoplasmic reticulum/cytosol, and AtGPX8 to the cytosol/apoplast (Rodriguez Milla et al., 2003; Margis et al., 2008) or the cytosol and nucleus (AtGPX8; Gaber et al., 2012). Phylogenetic analysis indicates that the AtGPX3 protein is localized to the endoplasmic reticulum/cytosol and that AtGPX6 is localized in the mitochondria (Margis et al., 2008). Each isoform within a given cellular compartment is considered to fulfil distinct functions. For example, AtGPX8 protects the nucleus and cytosol from the harmful effects of uncontrolled oxidation (Gaber et al., 2012). However, there has been no systematic characterization of the functions of the different GPX isoforms in Arabidopsis. Moreover, the precise roles of each GPX isoform in leaves and roots remain largely uncharacterized. The following experiments were therefore undertaken to explore the roles of the different AtGPX isoforms using gpx1, gpx2, gpx3, gpx4, gpx6, gpx7, and gpx8 T-DNA insertion mutant lines. A preliminary characterization of the different mutant lines revealed that the shoot phenotypes were largely similar in all genotypes, regardless of the GPX composition. Due to the absence of differences in shoot parameters between the gpx mutants and the wild type, the focus of this study was on the role of the different GPX isoforms in the control of root architecture, where significant differences were observed between the gpx mutants and the wild type. The aim of this study was to characterize the root phenotypes observed in the gpx mutants in relation to the action of hormones that control root architecture, particularly auxin, strigolactones (SLs), and abscisic acid (ABA).

### Materials and methods

**Identification and isolation of T-DNA insertion mutants**

T-DNA insertion lines from SAIL (Sessions et al., 2002), SALK (Alonso et al., 2003), and WiscDsLox (Woody et al., 2007) collections were obtained. Donor stock numbers SALK_027373 (gpx1; At2g259080), SALK_082445 (gpx2; At2g31570), SALK_278_E06 (gpx3-1; At2g43350), SALK_071176 (gpx3-2; At2g43350), SALK_139870 (gpx4; At2g48150), WISCDSLOX_321_H10 (gpx6; At4g11600), SALK_023283 (gpx7; At4g31870), and SALK_127691 (gpx8; At1g63460) were obtained from the ABRC (Ohio State University) and NASC (Nottingham Arabidopsis Stock Center). All T-DNA mutants except gpx3-1 are in the Col-0 background; gpx3-1 is in the Col-3 background. Plants were germinated and grown in conventional soil and, to confirm the position and the homozygosity of the insertion, PCR was performed with genomic DNA using gene-specific oligonucleotides whose sequences are given in Supplementary Table S2 available at JXB online. The position of the T-DNA insertion sequence and oligonucleotides for each genotype are shown in Supplementary Fig. S1 available at JXB online.

Upon request, all novel materials described in this publication will be made available in a timely manner for non-commercial research purposes, subject to the requisite permission from any third-party owners of all or parts of the material.

**Growth on soil and rosette measurements**

Wild types (Col-0 and Col-3) and gpx1, gpx2, gpx3-1, gpx6, gpx7, and gpx8 mutant seeds were grown on compost in controlled environment chambers under optimal growth conditions under either short (8 h) photoperiod or long (16 h) photoperiod conditions for 4 weeks. Rosette diameters were measured every 3 d. Leaf number, leaf fresh and dry weight measurements, and leaf pigment determinations were made on material harvested at 27/28 d.

**Growth on plates and hormone treatments**

Seeds of the wild type (Col-0) and gpx1, gpx2, gpx3-2, gpx4, gpx6, gpx7, and gpx8 mutants were surface sterilized for 2 min in 75% ethanol and 5 min in 4% sodium hypochlorite, and washed several times in sterilized water until the pH was ~7. Sterilized seeds were then sown on 12 cm square plates containing half-strength Murashige and Skoog medium (1/2 MS medium, pH 5.7) with 0.01%
myo-inositol, 0.05% MES, 1% sucrose, and 0.8% plant agar. Plates were stored for 2 d in the cold and dark to synchronize germination and were placed vertically in a plant growth cabinet with a 16 h photoperiod and a temperature of 22 °C. After 3 d, the seedlings were gently transferred using forceps to new plates containing the same medium plus 2 μM GR24 (synthetic SL) and grown for a further 5 d. Auxin treatments were performed by transferring the seedlings after a 5 d growth period to 1/2 MS medium containing 1 μM NAA (1-naphthaleneacetic acid) for 3 d. ABA was added to the media at concentrations of 0.3 μM or 0.5 μM. Seeds were placed directly on ABA-containing medium, cold treated, and grown as above for 8 d.

### Results

#### Relative abundance of AtGPX mRNAs in the gpx1, gpx2, gpx3, gpx4, gpx6, gpx7, and gpx8 mutants compared with the wild type

As a first step in the characterization of the functions of the different AtGPX isoforms, the relative abundance of the different AtGPX transcripts was compared in the roots and shoots of the gpx1, gpx2, gpx3-2, gpx4, gpx6, gpx7, and gpx8 mutants relative to the wild type (Col-0; Fig. 1; Supplementary Table S1 available at JXB online). AtGPX1 and AtGPX2 transcripts were abundant in shoots. The level of AtGPX2 transcripts was highest in roots, as was the level of AtGPX6 mRNAs (Fig. 1A). AtGPX4 mRNAs were below the level of detection in roots and shoots of the wild-type plants (Fig. 1A). AtGPX1 mRNAs were more abundant in the shoots of gpx3-2 and gpx4 mutants. The AtGPX2 transcripts were less abundant in the roots of the gpx7 mutants. AtGPX4 transcripts were less abundant only in the shoots of the gpx3-2 mutant. AtGPX5 mRNA levels were higher in the roots of the gpx1 and gpx4 mutants. AtGPX6 mRNA levels were lower in the root of the gpx7 mutant. AtGPX7 transcripts were increased in the shoots of the gpx4 mutants. AtGPX8 transcripts were less abundant in roots of gpx2, gpx3-2, and gpx7 mutants relative to the wild type. Gene expression data for each mutant are provided in Supplementary Table S1 available at JXB online.

#### Shoot phenotypes in the gpx1, gpx2, gpx3, gpx6, gpx7, and gpx8 mutants

In order to determine the importance of the different AtGPX isoforms for shoot parameters, the shoot phenotypes of the different mutants (gpx1, gpx2, gpx3-1, gpx6, gpx7, and gpx8) were compared with those of the wild type after 4 weeks growth under long (16 h) photoperiod growth conditions. Shoot biomass (fresh weight per plant) and leaf pigmentation (chlorophyll and carotene, expressed on a leaf area basis) contents were similar in all the lines (data not shown). However, the gpx2 and gpx8 mutants had significantly fewer leaves than the wild-type plants at the 28 d growth stage. In contrast, the gpx7 mutants had significantly more leaves than the wild type at this point (Fig. 2). To characterize the roles of the different AtGPX isoforms in shoot development further, shoot growth was also examined under short photoperiod (8 h) growth conditions. The shoot phenotypes of the mutants were very similar to those of their respective wild types when plants were grown under short day conditions (Fig. 3). However, gpx3-1 and gpx7 had a significantly greater rosette diameter than the wild type after 4 weeks under these conditions (Fig. 3C, E).

#### Root phenotypes in the gpx1, gpx2, gpx3, gpx4, gpx6, gpx7, and gpx8 mutants

To explore the roles of the different AtGPX isoforms in the control of root development, comparisons of the growth of the primary roots, the number of lateral roots, and LRDs were performed on 8-day-old seedlings (Fig. 4). Root phenotypes were compared in the different mutants (gpx1, gpx2, gpx3-1,
passaia et al. gpx3-2, gpx4, gpx6, gpx7, and gpx8) and the wild types (Col-0 and Col-3) in control conditions and in the presence of hormones. All the mutant lines showed large differences in LRD compared with the wild type in control conditions, with the gpx1, gpx4, gpx6, gpx7, and gpx8 mutants having greater LRD values relative to the wild type (Fig. 4C). In contrast, gpx2 and gpx3-2 had a lower LRD than Col-0 (Fig. 4C).

Auxin-dependent increases in lateral root density

In order to determine whether any of the different AtGPX isoforms were required for the auxin-dependent control of lateral root development, root phenotypes were compared in gpx1, gpx2, gpx3-2, gpx4, gpx6, gpx7, and gpx8 and Col-0 seedlings grown either in the absence of added hormone or in the presence of auxin (1 μM NAA; Fig 4). Auxin treatment resulted in a large increase in LRD in all of the genotypes. However, in contrast to the gpx2 and gpx3-2 mutants, which showed a response to auxin similar to that of the wild type (Fig. 4A–C), the effect of auxin was significantly greater in the gpx1, gpx4, and gpx7 seedlings than in the wild type.

Strigolactone (GR24)-dependent inhibition of lateral root development

The roles of the AtGPX isoforms in the SL-dependent control of lateral root development were investigated in the gpx1, gpx2, gpx3-2, gpx4, gpx6, gpx7, and gpx8 mutants. Col-0 and mutant seedlings were grown either in the absence of added hormone or in the presence of the synthetic SL, GR24 (2 μM; Fig. 4). As predicted from the known action of SLs on root architecture, the addition of GR24 inhibited lateral root formation in all the genotypes studied here. The addition of GR24 decreased LRD values to a similar extent in all the genotypes except for the gpx4 seedlings, which showed a significantly lower response to GR24-induced inhibition of LRD than the wild type (Fig. 4C).

Abscisic acid-induced inhibition of lateral root development

In order to determine whether any of the AtGPX isoforms were required for the ABA-dependent control of lateral root development, root phenotypes were compared in gpx1, gpx2, gpx3-1, gpx4, gpx6, gpx7, gpx8, Col-0, and Col-3 seedlings grown either in the absence of added hormone or in the presence of ABA (Fig. 5A–C). Growth in the presence of ABA decreased...
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Fig. 3. A comparison of rosette phenotypes in 4-week-old *gpx* mutants relative to the wild types grown under short day conditions. (A) *gpx1* mutant; (B) *gpx2* mutant; (C) *gpx3-1* mutant; (D) *gpx6* mutant; (E) *gpx7* mutant; (F) *gpx8* mutant and the respective wild types. Data are the mean ±SE (n=10). Asterisks indicate significant differences, *P*<0.05.
LRD in all the genotypes; *gpx*6 showed enhanced sensitivity to ABA-induced inhibition of lateral root development (Fig. 5).

**Effects of auxin on the developmental stages of the lateral root primordia**

The initiation and early development of lateral root is regulated by auxin. The activation of the lateral root primordium (LRP), which occurs in a well-defined spatial order, was compared in the mutant and wild-type lines. For simplicity, the early steps of LRP development were grouped into different stages that are designated I (initiation) to VIII (emergence), based on the cell layers formed (Malamy and Benfey 1997). The detailed comparisons of the different stages of primordia development are shown here only for the *gpx*2 mutants and Col-0 (Fig. 6). Auxin treatment significantly increased the number of primordia at developmental stages III–VIII in Col-0 and *gpx*2 (as indicated by asterisks), relative to controls grown in the absence of auxin (Fig. 6). The number of primordia of stages I–II was not altered by auxin treatment in either genotype. GPX2 deficiency did not alter the auxin-dependent development of LRPCs (Fig. 6).

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**Fig. 4.** A comparison of the effects of auxin and strigolactone on the root phenotypes in the different *gpx* mutants and the wild type. The primary root length (A), number of visible lateral roots (B), and lateral root density (C) were measured in seedlings of the different *gpx* mutant lines and the wild type (Col-0) grown in either the absence (black bars) or presence of auxin (1 μM NAA; grey bars) or strigolactone (2 μM GR24, light grey bars). Data are the mean ±SE. Symbols indicate significant differences, *P*<0.05 in control conditions (*); after auxin treatment (#); and after strigolactone treatment (§) in the mutants compared with the wild type.
Effects of strigolactones and auxin on the abundance of AtGPX transcripts

The effects of auxin (NAA) and SL (GR24) on the expression of AtGPX genes was characterized by comparing the effects of these hormones on the abundance of AtGPX transcripts in the roots and shoots of wild type seedlings. The addition of GR24 significantly increased AtGPX1 mRNAs in shoots and roots relative to controls (Fig. 7A, B) and increased the levels of AtGPX2 and AtGPX7 mRNAs in roots (Fig. 7A, B). The addition of auxin led to a significant increase in the abundance of all the AtGPX mRNAs, except for those of AtGPX1 and AtGPX7 in shoots (Fig. 7C). In contrast, AtGPX1 and AtGPX2 transcript levels were lower in the roots of the auxin-treated seedlings. However, AtGPX4 and AtGPX7 mRNA levels were higher in the roots after auxin treatment (Fig. 7D).
Discussion

GPXs are considered to play important roles in oxidative signalling and the metabolism of hydrogen peroxide, as well as the prevention of oxidant-induced PCD. The data presented here show that knockout mutations in each of the different GPX proteins had no adverse effects on leaf chlorophyll contents or rosette growth parameters. This finding would suggest that other components of the antioxidant system can compensate for the loss of GPX function in leaves in controlled growth conditions. Moreover, the gpx7 mutants had a greater rosette diameter than the wild type under shorter photoperiod growth conditions and more leaves than the wild type under long photoperiod growth conditions. These data suggest that GPX7 protein has functions in the pathways that constrain shoot development. In this regard, it is interesting to note that the addition of auxin increased the abundance of all of the GPX mRNAs, except for those of GPX7 and GPX1 in the shoots (Fig. 7). AtGPX7 and AtGPX1 are known to fulfil important roles in chloroplasts, contributing to the cross-talk
between high light stress and responses to pathogens (Chang et al., 2009). A part of this cross-talk involves the control of growth and development under optimal and stress conditions. The data presented here suggest a role for AtGPX7 but not AtGPX1 in the pathways that control shoot growth.

The systematic characterization of the functions of the different GPX isoforms in the control of shoot and root phenotypes reported here demonstrates the importance of these enzymes in the control of root architecture, particularly lateral root development. There is little information in the literature concerning the functions of GPX in roots. The roots of the gpx8 mutants are more sensitive to the oxidative stress induced by the herbicide methyl viologen (paraquat) than wild type roots (Gaber et al., 2012). The data reported here suggest that the GPXs are important in the hormone-mediated regulation of lateral root development. GSH is involved in the interplay between auxin and SL signalling that controls this process (Marquez-Garcia et al., 2013). For example, LRD was significantly decreased in GSH synthesis mutants (cad2-1, pad2-1, and rax1-1). Moreover, the application of GR24 increased the root GSH pool in a manner that was dependent on the MORE AXILLARY GROWTH (MAX) 2 signalling protein (Marquez-Garcia et al., 2013).

Auxin regulates the development of the LRP (De Smet et al., 2003). It triggers the first divisions of lateral root founder cells in the pericycle tissue of the primary root (Casimiro et al., 2003) and later it accumulates at the tip of the LRP, where it promotes the degradation of Aux/IAA proteins (Péret et al., 2009). Leaf-derived auxins are also important in the control of lateral root growth after emergence (Bhalerao et al., 2002; Swarup et al., 2008). The action of auxin is modulated by SLs, which influence auxin signalling pathways on multiple levels (Agusti et al., 2011). SLs inhibit lateral root initiation (Kapulnik et al., 2011) by blocking auxin transport (Bennett et al., 2006; Crawford et al., 2010; Balá et al., 2011). It has previously been shown that NADP-linked thioredoxin and GSH systems are required for auxin transport and signalling (Bashandy et al., 2010). Reduced thiols are also required for other processes that impact on root development. For example, the gat1 mutant (gfp-arrested trafficking), which is defective in thioredoxin m3, accumulates high levels of callose leading to the occlusion of the plasmodesmata in the root meristem, resulting in arrested root development (Benitez-Alfonso et al., 2009). The root phenotypes associated with the gpx mutants described here support the concept that redox processes involving glutathione and thioredoxins exert a strong influence on root development. GPXs may be required to mediate GSH and reduced thioredoxin functions in roots that impact on lateral root production or growth.

The data presented here demonstrate that GPX1 and GPX7 are important in the control of root architecture and lateral root development. Several plastid-localized enzymes that are important in the control of cellular redox homeostasis have also been shown to be required for root development. For example, the miao mutant is defective in the plastid-localized glutathione reductase (GR2) and consequently it has ~50% lower GR activity than the wild type and accumulates glutathione disulphide, GSSG (Yu et al., 2013). The miao mutant shows impaired root growth with severe defects in root meristem maintenance, suggesting that a high plastid GSH:GSSG ratio is important for root development (Yu et al., 2013). Conversely, severe GSH deficiency alone (without changes in the GSH:GSSG ratio) led to an arrest of the cell cycle at G2 in the root meristem with no apparent effect on root meristem maintenance (Schnaubelt et al., 2014). Less severe GSH deficiency or depletion of only the cytosolic GSH pool resulted in decreased root elongation and lower rates of lateral root formation (Kirchsteiger et al., 2012). The data presented here show that LRD values were higher in the gpx1 and gpx7 mutants than in the wild-type controls. Moreover, LRD values were more sensitive to auxin in the gpx1 and gpx7 mutants than in the wild type. These results strongly implicate the plastid-localized AtGPX1 and AtGPX7 enzymes in the auxin-dependent control of lateral root production. However, while GPX7 transcripts were significantly increased in roots as a result of the application of either auxin or SL, GPX1 transcripts were significantly more abundant following auxin treatment but significantly decreased in response to SL treatment. Hence, the hormone-dependent regulation of the AtGPX1 and AtGPX7 enzymes is different, at least at the level of gene expression. The gpx1 and gpx7 root phenotypes resemble the gstul7 mutant, which exhibits altered auxin-dependent regulation of lateral root development (Jiang et al., 2010). However, unlike the gstul7 mutants that show a lower sensitivity to ABA, gpx1 and gpx7 mutants show responses to ABA similar to those of the wild type.

In contrast to GPX1 transcripts, GPX2 mRNA levels were decreased in roots following the application of auxin but increased following the application of SL. However, the roots of the gpx2 mutants showed responses to SLs and to auxin very similar to those observed in the wild type, as did mutants deficient in other cytosolic GPX isoforms, such as gpx4. The gpx3 mutants had a lower LRD than the wild type but they showed similar responses to the wild type in root architecture to the auxin, SL, and ABA treatments. Like rice plants deficient in GPX3, which showed an altered root structure with shorter roots than the wild type (Passaia et al., 2013), the gpx3 mutants studied here had shorter primary roots and fewer lateral roots than the wild type. The AtGPX3 protein has been shown to be important in ABA signalling under drought stress (Miao et al., 2006). ABA suppresses the auxin response and is an inhibitor of lateral root development (De Smet et al., 2003). The abscisic acid insensitive 4 (abi4) mutant has an increased number of lateral roots relative to the wild type (Shkolnik-Inbar and Bar-Zvi, 2010). However, the responses of root architecture to ABA may vary between species because legume roots were shown to increase LRD in response to ABA (Liang and Harris, 2005). The data presented here show that roots of the gpx3 mutants had similar responses to the wild type control to ABA, indicating that AtGPX3 is not required for ABA-dependent control of root architecture.

The data presented here show that the application of auxin increased the abundance of several GPX transcripts. Auxin
is known to induce the expression of several glutathione S-transferases (GSTs) such as \textit{AtGSTF2}. \textit{AtGSTF2} binds indole-3-acetic acid (IAA) and the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA), and so enhanced expression of \textit{GSTF2} might be linked to stress-mediated growth responses (Smith et al., 2003). Mutants lacking another auxin-inducible GST, \textit{AtGSTU17}, were less sensitive to auxin and had lower numbers of lateral roots in the presence of auxin (Jiang et al., 2010). The \textit{gstu17} mutants were also less sensitive to ABA-mediated inhibition of root development (Jiang et al., 2010).

In conclusion, the data presented here demonstrate that the GPX proteins are important in the control of root architecture and that loss of any of the GPX isoforms exerts an influence on LRD. The plastid isoform \textit{AtGPX7} appears to be important in the control of shoot and root development by redox-dependent processes.

**Supplementary data**

Supplementary data are available at JXB online.

**Figure S1.** The positions of T-DNA insertions (blue arrows) in the \textit{gp} mutants and the positions of the primers used to detect the presence of the insert (black arrows).

**Table S1.** The relative expression of \textit{GPX} genes in different genotypes.

**Table S2.** Sequences of the primers used for the isolation of T-DNA insertion mutants (PCR: A and B) and for the gene expression analysis (qPCR; C).

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**References**


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