

Expression of a *Pteris vittata* glutaredoxin PvGRX5 in transgenic *Arabidopsis thaliana* increases plant arsenic tolerance and decreases arsenic accumulation in the leaves

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ABSTRACT

Chinese brake fern *Pteris vittata* hyperaccumulates arsenic in its fronds. In a study to identify brake fern cDNAs in arsenic resistance, we implicated a glutaredoxin, PvGRX5, because when expressed in *Escherichia coli*, it improved arsenic tolerance in recombinant bacteria. Here, we asked whether PvGRX5 transgenic expression would alter plant arsenic tolerance and metabolism. Two lines of *Arabidopsis thaliana* constitutively expressing PvGrx5 cDNA were compared with vector control and wild-type lines. PvGRX5-expressors were significantly more tolerant to arsenic compared with control lines based on germination, root growth and whole plant growth under imposed arsenic stress. PvGRX5-expressors contained significantly lower total arsenic compared with control lines following treatment with arsenate. Additionally, PvGRX5-expressors were significantly more efficient in their arsenate reduction *in vivo*. Together, our results indicate that PvGRX5 has a role in arsenic tolerance via improving arsenate reduction and regulating cellular arsenic levels. Paradoxically, our results suggest that PvGRX5 from the arsenic hyperaccumulator fern can be used in a novel biotechnological solution to decrease arsenic in crops.

Key-words: *Arabidopsis thaliana*; *Pteris vittata*; arsenate reduction; arsenic accumulation; arsenic tolerance; arsenic transport; glutaredoxin; heavy metal.

INTRODUCTION

The metalloid arsenic is a ubiquitous element in the environment and its occurrence results from both natural and man-made sources. Arsenic is a highly toxic class I human carcinogen. Chronic exposure to arsenic may lead to cancers of the skin, lung and internal organs and various other disorders (Shi, Shi & Liu 2004). Millions of people in various parts of the world are affected by arsenic contamination in drinking water (Shi *et al.* 2004). This public health

problem is most serious in Bangladesh, affecting more than 30 million people (Nordstrom 2002). In addition to this, in regions where irrigation water or soil is polluted with arsenic, accumulation of arsenic in harvestable parts of crops poses risk to food security (Queirolo *et al.* 2000; Meharg & Rahman 2003; Roychowdhury, Tokunaga & Ando 2003; Huang *et al.* 2006).

Ma *et al.* (2001) reported that brake fern *Pteris vittata* had an extraordinary ability to tolerate and hyperaccumulate arsenic, with its fronds accumulating arsenic up to 2.3% of their dry weight. Not only did this lead to the development of a cost-effective phytoremediation technology to use ferns to extract arsenic from contaminated soils and water (Caille *et al.* 2004; Tu *et al.* 2004; Kertulis-Tartar *et al.* 2006), but this also stimulated interest in arsenic metabolism in plants (Rathinasabapathi, Ma & Srivastava 2006a) and the use of ferns as a source of genes for stress tolerance (Rathinasabapathi 2006).

To better understand the genetic and biochemical mechanisms underlying arsenic resistance in *P. vittata*, we developed an efficient functional cloning method to rapidly identify *P. vittata* cDNAs based on their ability to increase arsenic resistance when expressed in *Escherichia coli* (Rathinasabapathi *et al.* 2006b). A cDNA for an unusual arsenate-activated glutaredoxin (PvGRX5) was implicated in arsenic metabolism because it improved arsenic resistance when expressed in *Escherichia coli* (Sundaram *et al.* 2008). Glutaredoxins are glutathione-dependent oxidoreductases known for their role in protecting specific proteins from damage by oxidative stress (Rouhier, Couturier & Jacquot 2006). Although plants contain a family of glutaredoxins whose functional roles are enigmatic (Rouhier *et al.* 2006), PvGRX5 is the first plant glutaredoxin linked to arsenate metabolism. Our analyses showed that PvGRX5 had a role in regulating cellular arsenite levels in recombinant bacteria (Sundaram *et al.* 2008).

Because bacteria and plants differ in their complexity and aspects of metabolism, it was important to directly test the role of PvGRX5 glutaredoxin in plants as well. The objective of the current study was to test whether

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constitutive expression of PvGRX5 would increase arsenic tolerance and alter arsenic metabolism in transgenic *Arabidopsis thaliana*.

MATERIALS AND METHODS

Plant material

Arabidopsis thaliana (ecotype Columbia) were grown individually in 3-in. plastic containers, using a commercial propagation medium (Mix number 2, Fafard Inc., Agawam, MA, USA). The plants were grown under 12-h-light photoperiod of $150 \mu\text{mol s}^{-1} \text{m}^{-2}$ supplied by cool white fluorescent lights at 24°C constant temperature. Upon reaching 5–10 cm length, the primary inflorescences were clipped once to favour the growth of multiple secondary bolts for plants to be used for *Agrobacterium*-mediated transformation (Clough & Bent 1998).

Construction of expression vector and *Agrobacterium*-mediated transformation

The open reading frame [726 base pairs (bp)] of the Pv5-6 cDNA clone (Genbank accession number EF052272) was amplified using the primers

5′–3′(GAGCTC)CGCCGGGGGAGAGAGAG and
5′–3′(GAGCTC)CCATCAAACACACGCCTTGAA.

The PCR product, cloned in pCR 2.1-TOPO vector to derive TOPO-PvGrx5, was verified by sequencing. TOPO-PvGrx5 was digested with *Sac*I and cloned in *Sac*I-linearized plant expression vector pMON-R5 (Fouad & Rathinasabapathi 2006) to derive pMON-R5-SG2. The orientation of the insert was confirmed by restriction analysis. The pMON-R5-SG2 and pMON-R5 were transferred into *Agrobacterium tumefaciens* ABI strain via tri-parental mating (An *et al.* 1988). Plants were inoculated with *A. tumefaciens* strain (ABI), carrying pMON-R5-SG2 or pMON-R5 by the floral dip method (Clough & Bent 1998). The aerial floral parts of the plants were dipped for 30 s in 100 mL of 0.5X Murashige and Skoog (MS) medium (Murashige & Skoog 1962) containing 5% (w/v) sucrose with *Agrobacterium* cells at 0.5 A_{600} and 0.03% (v/v) of the surfactant Silwet L-77 (Union Carbide, Danbury, CT, USA). After the inoculation, plants were covered with a transparent plastic dome to maintain humidity for 24 h and then returned to the growth chamber till seed set. Putative transformants (T1) were selected based on their resistance to kanamycin (50 mg L^{-1}) in the half-strength MS medium with 0.8% (w/v) agar. Further, the presence of the transgene was verified using a PCR procedure on isolated genomic DNA as a template with PvGrx5 gene-specific primers. The positive plants were grown in controlled environments and the seeds were collected from individual plants. From the T2 seeds, segregating for the transgene 3:1, several plants were selfed and their progeny analysed for homozygosity. Homozygous T3 lines identified from these analyses were used for evaluations of transgene expression and arsenic tolerance.

Evaluation of arsenic tolerance

Arsenic resistance during germination was evaluated by plating the transgenic seeds in half strength MS medium containing 2% (w/v) sucrose and 0.8% (w/v) agar supplemented with or without $50 \mu\text{M}$ sodium arsenite. The seeds were surface sterilized using 20% (v/v) commercial bleach and by washing thoroughly with sterilized water. Approximately 100 seeds were plated and then kept at 4°C for 48 h, and then at 23°C for 3 d under light ($70 \mu\text{mol m}^{-2} \text{s}^{-1}$) for calculating the germination rate under control and the arsenic treatment conditions.

For root growth evaluations, 3-day-old seedlings germinated on half strength MS agar medium were transferred to vertical plates containing half-strength MS agar medium with 1 mM sodium arsenate or $25 \mu\text{M}$ sodium arsenite. By marking and measuring the root tip at the beginning and end of a 48 h period, root growth rate was calculated.

To evaluate whole plant arsenic tolerance, PvGRX5-expressing transgenic (SG2 lines), wild type (WT) and the vector control (VC) were grown in individual containers under identical conditions. One-month-old plants were irrigated with or without sodium arsenate in half-strength Hoagland nutrient solution (Hoagland & Arnon 1938). The arsenic treatment was provided using stepwise increase of arsenate from $321 \mu\text{M}$ up to $3210 \mu\text{M}$ over a 14 d period. The arsenic-containing medium was added to the potting medium without touching the aerial portion of the plants. Growth difference was observed by measuring the above-ground biomass fresh weight and dry weight after completely drying the samples at 80°C .

Arsenic speciation

Known fresh weight of leaf tissue was extracted in 50% (v/v) methanol in water (Rathinasabapathi *et al.* 2006b). Arsenate and arsenite were separated using an arsenic speciation cartridge (Metal Soft Center, Highland Park, NJ, USA), which retains arsenate. Total arsenic and arsenite were determined by using an atomic absorption spectrophotometer (Varian 240Z, Zeeman Atomic Absorption Spectrophotometer, Varian, Walnut Creek, CA, USA) by the method of Chen & Ma (1998).

Photosynthetic rate measurements

Light-saturated net photosynthesis was measured using a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA). Conditions within the sample chamber were: $[\text{CO}_2] = 370 \mu\text{mol mol}^{-1}$, block temperature = 23°C and photosynthetic photon flux = $75 \mu\text{mol m}^{-2} \text{s}^{-1}$. Net photosynthesis rates are expressed as rates of CO_2 uptake ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$).

Glutaredoxin assay

Soluble protein was extracted from leaves by the method of Fouad & Rathinasabapathi (2006). Glutaredoxin activity

was determined with a coupled enzyme reaction (Holmgren 1989). In this assay, NADPH-dependent reduction of 2-hydroxyethyl disulfide (HED) in the presence of glutathione reductase (GR) was monitored at 340 nm using a UV-visible spectrophotometer (Beckman Coulter, Fullerton, CA, USA). The assay mixture contained 100 $\mu\text{g mL}^{-1}$ of bovine serum albumin, 1 mM GSH, 6 $\mu\text{g mL}^{-1}$ yeast GR, 0.4 mM NADPH, 0.1 M Tris-HCl, 2 mM ethylenediaminetetraacetic acid, pH 8.0, and 0.7 mM HED in a total volume of 800 μL . Non-enzymatic NADPH-dependent reduction of HED was measured for the background. Total protein was estimated by a modified Lowry method (Peterson 1977). The enzyme activity was expressed as $\text{nmol mg}^{-1} \text{ protein min}^{-1}$.

Quantitative reverse transcriptase-PCR (QRT-PCR)

Transcript quantification was done by using 7900 HT fast real-time PCR system (Applied Biosystems, Foster city, CA, USA). Total RNA, isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used for first strand cDNA synthesis. cDNA synthesis was done by using superscriptTM first strand synthesis system (Invitrogen). Then this was used as template for quantifying the transcript level of PvGrx5 using the gene-specific primers (qgrxF-5'-ATG GCA TCC AGG GCC GTG CAA-3' qgrxR-5'-CAA TTC CGG TGA CAA ACC AGT TGG G -3') using DyNAmoTM HS SYBR[®] Green qPCR kit (New England Biolabs, Beverly, MA, USA). Primers for an actin were included as an internal control. Following a pre-incubation of the mixture at 50 °C for 2 min and 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min were used, followed by 95 °C for 15 s and 60 °C for 15 s. Relative shift in the threshold cycle (ΔCt) value was determined by subtracting the Avg ΔCt of Grx – Avg ΔCt of actin ($n = 3$).

Determination of pigments

Pigments were extracted using 80% (v/v) ice-cold acetone from the leaf tissue and chlorophyll *a* and *b*, carotenoids and anthocyanins were estimated using an ultraviolet-visible spectrophotometer (Beckman Coulter, Fullerton, CA, USA) by the method of Lichtenthaler and Wellburn (1983) and Harborne (1998), respectively.

Arsenate reduction *in vivo*

To determine arsenate reduction, uniform leaves (about 50 mg fresh weight), excised from month-old plants were supplied with 1, 10 and 30 mM sodium arsenate solutions as described in Rathinasabapathi *et al.* (2006b) and incubated under light for 3 h. The tissue samples were extracted in 50% (v/v) methanol in water and total arsenic, arsenite and arsenate were determined as described above. The actual amounts of arsenate infiltrated were determined from the total arsenic values in the tissue extracts. The rate of arsenate reduction was calculated by determining μg of arsenite

per hour per gram tissue and plotted against amounts of infiltrated substrate. Apparent K_m and V_{max} values were determined using a Lineweaver–Burk plot (Segel 1993).

Data analysis

Quantitative data were analysed using analysis of variance using a PC-based SAS programme (SAS Institute 1997). Mean separations were done by using Duncan's multiple range test at $P = 0.05$. All values reported in this work are means of at least five individual replications, unless mentioned otherwise. Experiments were repeated two to five times. Results were expressed as means followed by corresponding standard errors.

RESULTS

Expression of PvGrx5 cDNA in transgenic *A. thaliana*

PvGrx5 cDNA was expressed in transgenic *A. thaliana* under the control of a constitutive modified FMV promoter. QRT-PCR analyses of leaf RNA using gene-specific primers indicated that the transgene was expressed at varying levels in the four SG2 homozygous lines with the decreasing order of: SG2-7 > SG2-1 > SG2-B > SG2-5 with no detectable transcripts in the vector control and wild-type (Fig. 1a). When total glutaredoxin activity was measured in leaf protein extracts, the specific activities in all of the four SG2 lines were greater than that found in wild-type and vector control (Fig. 1b). Two lines SG2-1 and SG2-7 exhibiting the highest glutaredoxin-specific activities, were taken up for further evaluations and compared with wild-type and vector control lines.

Arsenic tolerance of control and PvGRX5-expressing lines

In a germination test on arsenite-containing medium, seed germination of vector control and wild-type lines were significantly inhibited but PvGRX5 expressors SG2-1 and SG2-7 were not affected (Fig. 2a). A root growth bioassay indicated that root growth of SG2 lines was less sensitive to inhibition by sodium arsenate and sodium arsenite than wild-type and vector control lines (Fig. 2b).

Following these *in vitro* assays done on germinating and young seedlings, whole plant sensitivity to arsenate was investigated using 1-month-old container-grown plants. Under control conditions, the biomass per plant was not significantly different between vector control, wild-type and SG2-1, although SG2-7 was significantly greater than all other lines (Fig. 3b). Under arsenic stress, growth of wild type and vector control was significantly reduced compared with control conditions (Fig. 3a,b), but PvGRX5-expressing SG2 lines were not inhibited (Fig. 3b). When carbon dioxide fixation was measured, it was significantly less in vector control plants exposed to arsenic but not in wild-type and SG2 lines (Fig. 4a). Estimations of leaf anthocyanin content indicated that under arsenic stress, vector control and

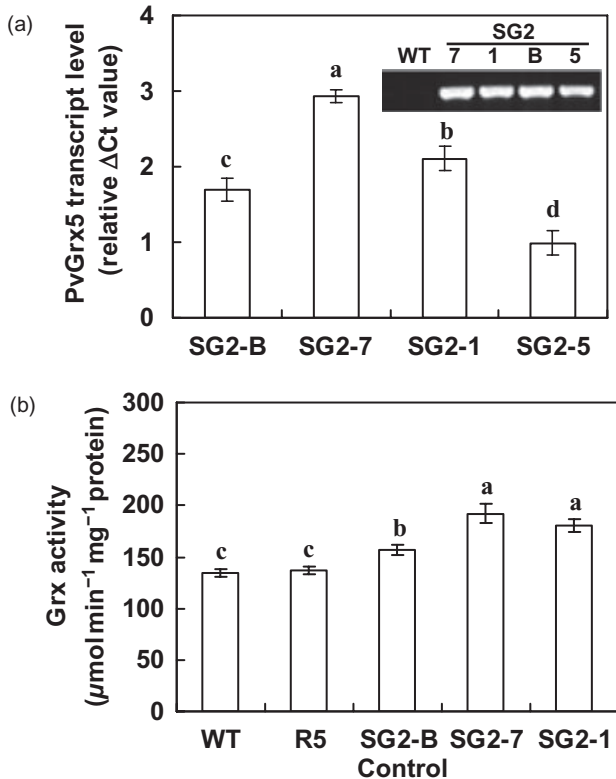


Figure 1. Expression of *Pteris vittata* glutaredoxin in transgenic *Arabidopsis thaliana*. (a) PvGrx5 transcript levels were measured by quantitative RT-PCR. Inset: PCR amplification of PvGrx5 using genomic DNA as a template from the SG2 lines and not wild-type (WT). (b) Extractable glutaredoxin specific activities from leaves of SG2 lines and vector control (R5) and wild-type (WT) lines. Bars representing means and standard errors, marked with the same letter, are not significantly different using Duncan's multiple range test ($P \leq 0.05$). Δ Ct, shift in the threshold cycle.

wild-type lines had significantly more anthocyanin than the SG2 lines (Fig. 4b).

To test whether whole plant arsenate tolerance was correlated to active PvGRX5, glutaredoxin activity was measured in soluble leaf protein fractions from plants exposed to arsenate. PvGRX5-expressing lines had significantly increased glutaredoxin-specific activities compared with control lines (Fig. 5a).

To test the fate of arsenic in PvGRX5-expressing lines, total arsenic, arsenate and arsenite levels in leaves were determined following exposure of plants to arsenate in the irrigation medium. Compared with vector control and wild-type, PvGRX5-expressing line SG2-7 had significantly lower total arsenic (Fig. 5b). Although arsenite level in SG2-1 was significantly lower than in wild-type and in vector control, arsenate levels between wild-type and SG2-1 were not significantly different (Fig. 5b).

To test the rate of arsenate reduction *in vivo*, leaf tissues of vector control and PvGRX5-expressing line SG2-7 were supplied with sodium arsenate by vacuum infiltration. Following incubation under light for 3 h, arsenic in the tissue

was analysed. The results showed that arsenate reduction rate in the PvGRX5-expressing line was significantly greater than in the vector control ($P > 0.05$) at the lowest substrate concentration tested (Fig. 6a and 6a inset). A kinetic analysis of the data showed that the V_{max} values were not significantly different between the vector control line and SG2-7 line, but SG2-7 line had a lower apparent K_m value than the vector control [0.324 and 0.773 mM As(V) for PvGRX5 and vector control, respectively, Fig. 6b].

DISCUSSION

In a previous study, we showed that *P. vittata* glutaredoxin PvGRX5 had a specific role in arsenic tolerance, fitting this fern's relatively high cellular levels of glutathione and oxidative stress tolerance (Singh *et al.* 2006). PvGRX5-expressing recombinant *E. coli* had greater tolerance to arsenate and arsenite than the vector control but recombinant *E. coli* expressing bacterial glutaredoxin did not (Sundaram *et al.* 2008). Given the current lack of gene

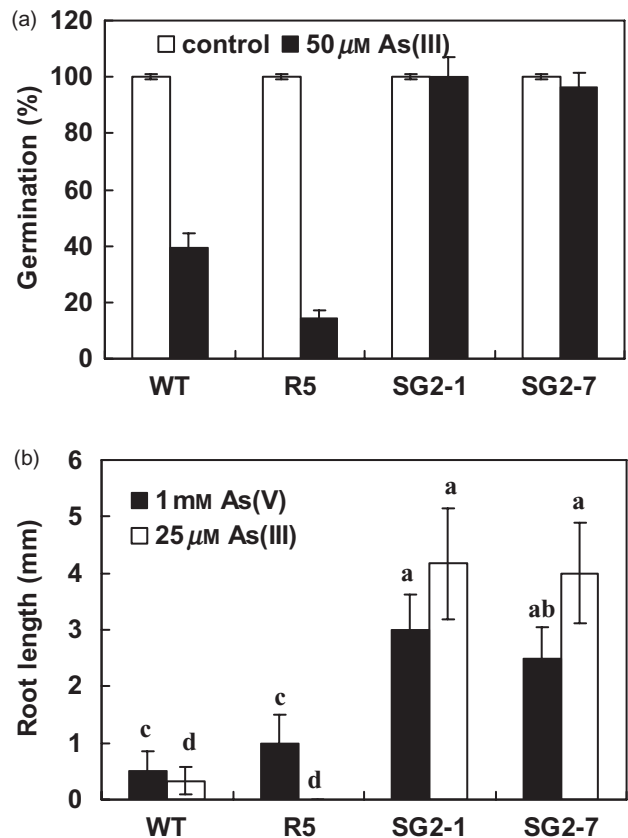


Figure 2. Arsenic tolerance of seedlings expressing *Pteris vittata* glutaredoxin PvGRX5. (a) Germination of wild-type (WT), vector control (R5) and two SG2 lines on medium containing no (open bars) or 50 μ M sodium arsenite (dark bars). (b) Rate of root growth on medium containing 1 mM sodium arsenate (dark bars) or 25 μ M sodium arsenite (open bars). Bars representing means and standard errors, marked with the same letter, are not significantly different using Duncan's multiple range test ($P \leq 0.05$).

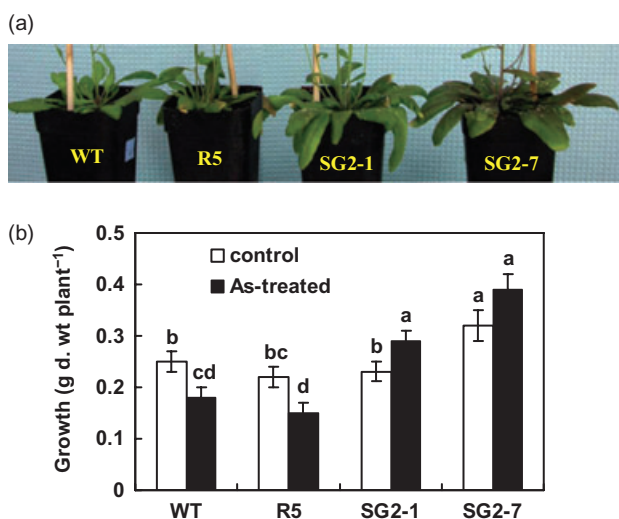


Figure 3. Arsenate tolerance of *Arabidopsis thaliana* expressing PvGRX5 (SG2) compared with wild-type (WT) and vector control (R5). (a) Morphology and (b) growth, following a 2-week treatment with arsenate in the irrigation medium. Bars representing means and standard errors, marked with the same letter, are not significantly different using Duncan's multiple range test ($P \leq 0.05$). d. wt, dry weight.

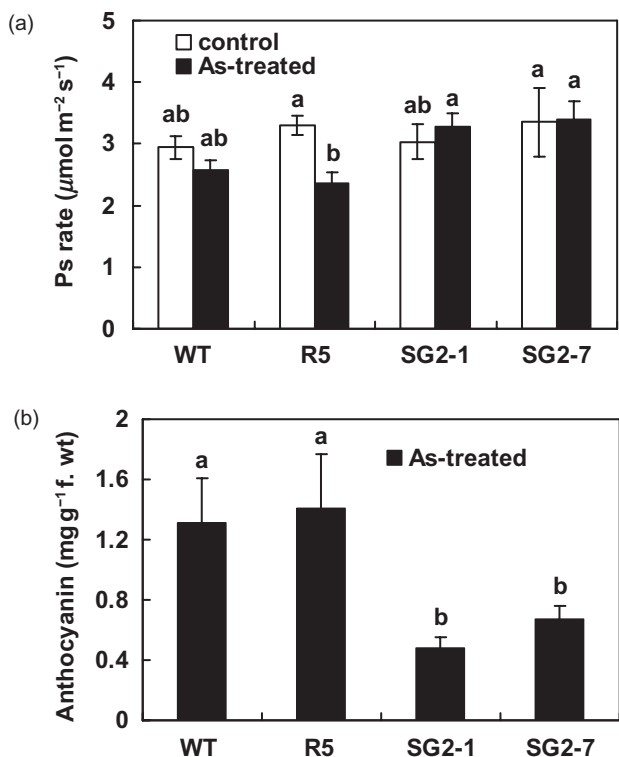


Figure 4. Arsenate tolerance of *Arabidopsis thaliana* expressing PvGRX5 (SG2) compared with wild-type (WT) and vector control (R5). (a) photosynthetic carbon fixation rate and (b) anthocyanin levels, all following a 2-week treatment with arsenate in the irrigation medium. Bars representing means and standard errors, marked with the same letter, are not significantly different using Duncan's multiple range test ($P \leq 0.05$). f. wt, fresh weight.

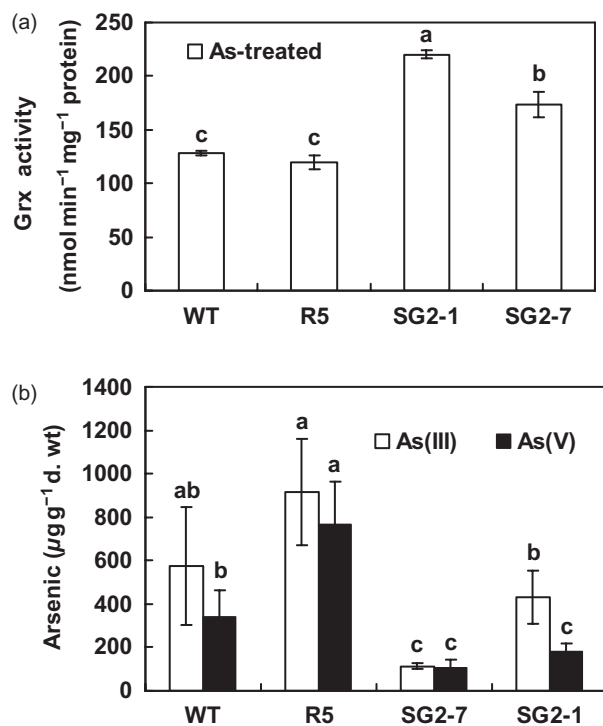


Figure 5. PvGRX5 expressing lines have lower arsenic levels. (a) Glutaredoxin-specific activities in leaves from plants exposed to arsenate in the irrigation medium (b) Arsenite (open bars) and arsenate (filled bars) levels in wild-type (WT), vector control (R5) and SG2 lines. Bars representing means and standard errors, marked with the same letter, are not significantly different using Duncan's multiple range test ($P \leq 0.05$). d. wt, dry weight.

transfer methods in this fern, we chose to utilize a heterologous transgenic expression method to evaluate the function of PvGRX5 in plants.

Construction of four independent homozygous transgenic lines expressing PvGRX5 at different levels made it possible to show that constitutive expression of PvGRX5 was not detrimental to normal plant growth and development (Fig. 1). Our data show that transgenic expression of PvGRX5 in *A. thaliana* was sufficient to increase plant tolerance to arsenic (Figs 2 & 3).

Based on growth and photosynthesis measurements, PvGRX5-expressing lines SG2-1 and SG2-7 were not affected by the arsenate treatment applied (Figs 3 & 4). Upon visual inspection, the plants appeared to be differing in their red colouration in the leaves. Pigment analyses on leaves of comparable developmental stages revealed that SG2 lines did not differ from control lines for chlorophyll content (data not shown) but they had significantly less anthocyanin than the control lines under arsenic stress (Fig. 4b). This is consistent with anthocyanin synthesis as a visible marker of arsenic stress (Catarchea *et al.* 2007).

Glutaredoxin-specific activities were correlated to whole plant tolerance to arsenate (Fig. 5), consistent with PvGRX5's role in arsenic metabolism. Although 31 glutaredoxins are known in *Arabidopsis* (Rouhier *et al.* 2006), addition of arsenate-inducible PvGRX5 (Sundaram *et al.*

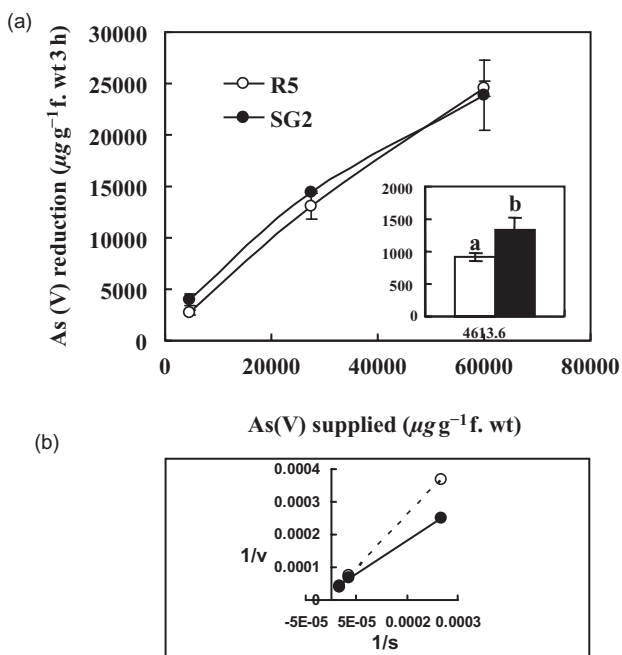


Figure 6. Arsenate reduction rate *in vivo*. (a) Arsenate reduction rate in leaf tissues incubated with sodium arsenate for 3 h, at different As(V) concentrations, as determined by extracted total As in the samples. The inset shows per hour rates at the lowest concentration. (b) Lineweaver–Burk plot for data shown in (a). Data for vector control and SG2-7 line are shown with empty and filled circles, respectively. Data points show mean and standard error ($n = 3$). f. wt, fresh weight.

2008) in SG2 lines reduced their sensitivity to arsenate than that found in the wild-type and vector control lines.

To understand the role of PvGRX5 in arsenic tolerance in SG2 lines, we measured the arsenic content and species in the leaves following treatment with arsenate in the irrigation medium. PvGRX5-expressing lines had significantly lower total arsenic in the leaves (Fig. 5b) than the vector control line. This function of PvGRX5 was also observed in recombinant *E. coli* expressing PvGRX5, where genetic evidence suggested that it interacted with an aquaglyceroporin with a role in arsenite efflux (Sundaram *et al.* 2008). Work by others show that arsenite efflux can be altered in plants by novel mechanisms. For example, genetic evidence is available for the role of a tobacco protein with a ribosomal domain in arsenite efflux (Mok *et al.* 2008). Alternatively, lower levels of arsenic in the leaf in PvGRX5-expressors could be due to lower arsenic translocation from the roots to the shoots. The lines we have generated here will be useful in future mechanistic investigations to find PvGRX5's interacting protein, and whether PvGRX5 expression affects arsenate uptake, arsenic translocation and efflux.

In micro-organisms, glutaredoxin has been shown to be an electron donor for arsenate reductases (Shi *et al.* 1999; Mukhopadhyay, Shi & Rosen 2000). Plants, including *P. vittata* and *Arabidopsis*, express glutaredoxin-dependent arsenate reductases (Dhankher *et al.* 2006; Ellis *et al.* 2006), although specificity of plant glutaredoxins in this role is not

clearly understood. Because it is possible that PvGRX5's role in arsenate tolerance is via increased arsenate reduction, we tested *in vivo* arsenate reduction rates. Leaves from SG2-7 line reduced arsenate at a greater rate than the control line at low arsenate concentrations (Fig. 6a and 6a inset). Kinetic analyses showed that the apparent K_m for arsenate was lower in the PvGRX5-expressing line than in the vector control (Fig. 6b). These results suggest that *A. thaliana*'s native glutaredoxins are not limiting for arsenate reduction but the additional expression of PvGRX5 improved the kinetics of the reaction, making it more efficient. Future studies should examine this aspect using purified *A. thaliana* arsenate reductase, wherein purified *Arabidopsis* and fern glutaredoxins can be compared for kinetic efficiencies.

Despite its source from an arsenic hyperaccumulator plant, transgenic expression of PvGRX5 reduced arsenic content in plants exposed to arsenic. Although the mechanistic details behind this paradox are not completely clear, it is likely that there are several independent and interacting genes for arsenic tolerance and arsenic hyperaccumulation in *P. vittata*. As elucidated here, PvGRX5's role could be in cellular arsenic tolerance and homeostasis. Other factors, including gene regulation and interactions between members of gene networks could be different between *P. vittata* and PvGRX5-expressing transgenic plants. Irrespective of these possibilities, our results strongly suggest that PvGRX5 could be used to engineer transgenic crops with an ability to accumulate low arsenic levels in the leaves and, consequently, also in other edible parts. This is especially significant in the light of controlled experiments indicating that when crop plants were irrigated with arsenic-containing medium, leaves accumulated significantly greater amounts of arsenic than the grains (Abedin, Cotter-Howells & Meharg 2002). The potential utility of the gene-encoding PvGRX5 to achieve both arsenate tolerance and low arsenic accumulation is timely, as marker-assisted selection work indicated that arsenic accumulation in rice is a complex quantitatively inherited trait (Zhang *et al.* 2008).

Many others have altered arsenic tolerance in transgenic plants via over-expressing specific genes-encoding enzymes involved in the synthesis of phytochelatin, the thiol peptides known in heavy metal detoxification (Dhankher *et al.* 2002; Li *et al.* 2004, 2006; Gasic & Korban 2007). However, the characterization of PvGRX5 represents the first successful attempt towards a genetic dissection of arsenate tolerance mechanism in the arsenic hyperaccumulator fern and is likely independent of phytochelatin.

The transgenic plants generated in this work will be unique material to investigate the potential roles of glutaredoxins in arsenic uptake, transport and efflux. Studies on the evolution of PvGRX5 and related glutaredoxins should help us understand the evolution of arsenic tolerance in ferns.

ACKNOWLEDGMENTS

This work was supported by funds to B.R. from the USDA TSTAR (2005-34135-15898) and the Florida Agricultural

Experiment Station. We thank Dr Uttam Saha for help with arsenic speciation and Dr Charles Guy for useful discussions.

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Received 21 November 2008; received in revised form 3 February 2009; accepted for publication 4 February 2009