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Differential Expression in Response to Biotic and Abiotic Stress from Three Potato Glutaredoxins Induced during Suberization

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To determine their tissue-specific expression, three GRX genes were isolated from ESTs of a "6-hr post-suberization" cDNA library for *Solanum tuberosum* L. cv. Desiree. Transcripts of a CCxC/S type GRX, StGRX1, accumulated to higher levels during the vegetative-growth stage in the leaves, stem, and roots. In contrast, transcripts of a CxxC/S type GRX, StGRX2, accumulated in the reproductive tissues, such as the flower and tuber. Differential expression of these three potato StGRXs suggest specific and unique physiological roles during plant development and in response to environmental cues. Therefore, we propose that StGRX1 is a housekeeping gene, functioning in the rapid defense against various abiotic and biotic stresses, while StGRX2 and -3 are selectively expressed during growth and in response to internal and external signals.

Key words: biotic and abiotic stresses, expression, glutaredoxin, potato

Glutaredoxins (GRXs) are ubiquitous small proteins in the thioredoxin (TRX) family that catalyze dithiol-disulfide exchange reactions or reduce protein-mixed glutathione disulfides. In bacterial, yeast, and mammalian cells, GRXs appear to be involved in maintaining cellular redox homeostasis. However, in plants, their physiological roles have not been fully characterized. Proteins belonging to the TRX superfamily contain a reactive Cys pair that undergoes reversible disulfide bond formation as electrons are transferred to a variety of reductases (Holmgren, 1989). Thioredoxin specifically is reduced by NADPH or ferredoxin-dependent thioredoxin reductase. GRX, a related cofactor, is specifically reduced by glutathione (GSH), which is maintained in the reduced state by NADPH-dependent glutathione reductase. This specificity for GSH lies in a GRX glutathione-binding site, which is absent from thioredoxin (Nikkola et al., 1991). GRXs, like TRXs, may operate as dithiol reductants, and perform fast but reversible thiol-disulfide exchange reactions among cysteines in the active site and between cysteines and their disulfide substrates. Additionally, GRXs uniquely reduce mixed disulfides via a monothiol mechanism (Fernandes and Holmgren, 2004).

Studies of higher plants have focused on TRX, while very little is known about GRX even though cDNA sequences have long been available (Minakuchi et al., 1994; Szederkenyi et al., 1997). In most organisms, TRXs and GRXs are the major reducing molecules, and are involved in many cellular processes. Recent genomic analyses (Lemaire, 2004; Rouhier et al., 2004) have identified three types of GRXs -- CPYC, CGFS, and CC – the last subgroup being composed of a GRX with a CCXC or CCXS active site, and seemingly specific to higher plants. In *Arabidopsis*, 31 GRX genes have been identified. Six are of the classical CPYC type, intensively studied in *E. coli* and yeast, while four are of the CGFS type, which, thus far, have been analyzed in yeast and humans. As in *E. coli*, yeast, and humans, GRXs with active sites of the CPYC and CGFS types also are found in lower and higher plants, although little is known about their functioning there. Surprisingly, 21 GRXs from *Arabidopsis* contain a novel, plant-specific CC-type motif.

Information is accumulating on the role of CC-type GRXs and the regulation of cellular redox status. GRXs are abundant in the phloem sap, as are TRX and peroxiredoxin (PRX) (Balachandran et al., 1997; Szederkenyi et al., 1997; Rouhier et al., 2001). To date, biochemical data have been obtained only from a poplar CPYC-type GRX, where it participates in oxidative stress responses, reducing a Type II PRX (Rouhier et al., 2001, 2002). ROXY1, a CC-type glutaredoxin, also is involved in petal development in *A. thaliana* (Xing et al., 2005). However, the targets of GRX remain largely unknown. Recently, glutaredoxin targets have been identified in photosynthetic organisms, using affinity columns made with mutated GRX (a classical GRX with an active-site sequence mutated into CPYS) from poplar. This strategy is similar to those adopted for finding TRX targets, and has allowed for the identification of 94 putative GRX targets from different higher plant tissues (Rouhier et al., 2005). However, most targets previously identified had already been retained on monocysteinic TRX columns, illustrating again the difficulty in using such approaches to identify targets that are specific for GRX.

When plants are wounded, they form a protective layer next to the exposed surface to prevent dehydration and potential penetration by opportunistic pathogens. This physical barrier, i.e., suberin, comprises a specific cell wall modification characterized by both a polyphenolic domain and a wax-embedded polyaliphatic domain (Bernards and Lewis, 1998; Bernards, 2002; Razem and Bernards, 2003). Most studies of the composition and structure of suberin and associated materials have been made using the potato wound-induced periderm (Kolattukudy and Agrawal, 1974; Stark and Garbow, 1992) because of its potential for quick and uniform suberization in response to wounding. The polyphenolic domain has been likened to lignin, which is a three-dimensional polymeric network derived from the monolignols (*p*-coumaryl, conniferyl, and sinapyl alcohols) plus a significant amount of hydroxycinnamic acids and their

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derivatives (Bernards and Razem, 2001; Bernards, 2002). The biosynthesis of this domain is hypothesized to follow a peroxidase-mediated oxidative coupling process. Rapid induction of reactive oxygen species (ROS) under stress conditions initially results in the production of superoxide anions. Although the uncontrolled formation of ROS can cause extensive damage to plant cells, it remains a crucial element during biotic and abiotic stress responses, especially in plant-microbe interactions and wound-induced responses. To maintain an appropriate intracellular redox environment that minimizes the toxicity of ROS, efficient redox and antioxidant systems are required, such as for glutathione and thioredoxin redox.

Here, we investigated the roles of potato glutaredoxins during the suberization process and under various stresses. We used *in silico* screening of three different GRX sequences from ESTs of a "6-hr post-suberization" cDNA library to isolate cDNAs and examine their functioning.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

Plants of *Solanum tuberosum* L. cv. Desiree were vegetatively propagated from nodal stem segments on an MS medium containing 3% (w/v) sucrose, at 24°C and under a 16-h photoperiod. Leaves and stems collected from these plants were used in our expression studies. For *in vitro* tuberization, microtubers were produced by the method previously reported by Jeon et al. (1992), with minor modifications. Nodal cuttings taken from shoot cultures were placed on the medium described above, but with a sucrose concentration of 90 g L^{-1}. These were placed in a growth room maintained at 18°C. To induce biotic and abiotic stress, samples cut at the fourth node from each cultured shoot were first transferred from their Petri dishes to a liquid vial system. Tubers harvested from greenhouse cultivation were used for suberization tests.

Construction of The cDNA Library and Sequencing

Field-grown potato tubers were sampled for the extraction of total RNA, which was isolated from the fleshy part at 6 h after suberization, according to the Phenol/SDS method (Ausubel et al., 1987). Approximately 5 μg of mRNA was used to synthesize cDNA, which was then ligated with an adaptor of the EcoRI and XhoI restriction enzyme sites for directional cloning into the Uni-ZAP XP vector with a cDNA library kit (Stratagene). Approximately 1200 pBluescript phagemid with the cloned DNA insert were released from the Phagemid library according to the manufacturer's protocol (Stratagene). The cDNA inserts were sequenced with a T3 primer (5'-AAT TAA CCC TCA CTA AAG GG-3'), using a DYEnamic ET Terminator cycling sequencing kit (Amersham Biosciences) on an automated sequencer (RISA384, Shimadzu). In total, 1072 ESTs were isolated and analyzed from 1124 sequenced data, showing 173 clusters and 630 singlets that represented unique sequences. Nucleotide and amino acid sequences were aligned via ClustalW and analyzed with an NCBI Blast X search (Altschul et al., 1997).

Biotic and Abiotic Stress Conditions

We used a common assay (Doke, 1975) to assess the responses of potato plants to the pathogen *Phytophthora infestans*. Plantlets cultivated in pots for one month were inoculated with 20 mL of a sporangia suspension ($1x10^3$ sporangia mL^{-1}) on the centers of the abaxial leaf surfaces. These inoculated plantlets were then kept for 3 d in an incubator set at 21°C and 100% humidity. DNA probes were PCR-amplified from the PR-2 and -3 genes of pepper cDNAs selected from the EST database (http://plant.pdrc.re.kr/ks200201/pepper.html). For suberization tests, tubers were cut with knife about 1 cm thick sections and incubated at room temperature in the dark for 0, 6, 12, 24, 36, 72, and 120 h intervals after wounding. For salt stress, 5-day-old shoots in vials were transferred for 24 h to subculture media containing 0, 100, 200, or 300 mM NaCl. To create osmotic stress, 5-day-old shoots in vials were transferred for 24 h to subculture media containing 0, 100, 200, or 400 mM mannitol. Dehydrin probes were PCR-amplified from cDNA of the potato dehydrin gene (GenBank Accession No. AAB53203). For low-temperature stress, 5-day-old shoots in vials were incubated in an icebox (0°C) for 0, 1, 3, 6, 12, or 24 h. For ethephon treatment, 5-day-old shoots in vials were transferred for 0, 6, 12, 24, or 48 h to a subculture medium containing 25 mg L^{-1} ethephon. For hormone treatments, 5-day-old shoots in vials were transferred for 0, 1, 3, 6, 12, or 24 h to subculture media containing GA_3, zeatin, ABA, or MeJA (each at 1 mg L^{-1}). Plant materials exposed to these different stress treatments were then collected and frozen in liquid nitrogen.

Southern and Northern Blot Analyses of Three Potato StGRXs

Southern blot analysis was performed on genomic DNA extracted from potato leaves, following a previously reported protocol (Rogers and Bendich, 1988). After digestion with the appropriate restriction enzymes, DNA was subjected to electrophoresis through a 0.7% agarose gel before being transferred to a nylon membrane. Three different GRX probes were generated by PCR, using cloned potato cDNAs as the template, with primers designed from the sequences of the potato GRX1, 2, and 3 genes (GenBank Accession No. EF635995, EF635996, and EF635997, respectively). The membranes were hybridized with probes labeled with digoxigenin (DIG), using a PCR DIG Probe Synthesis Kit (Roche Molecular Biochemicals). Hybridization in DIG Easy Hyb buffer lasted for 16 h at 42°C. The membranes were then washed twice in 2X SSC, 0.1% (w/v) SDS at room temperature for 5 min each time, and then twice in 0.1X SSC, 0.1% SDS at 68°C for 15 min each time. Target DNAs were detected with a DIG luminescence detection kit, according to the manufacturer's instructions (Roche Molecular Biochemicals). Total RNA was isolated from samples using the above-mentioned Phenol/SDS method. In all, 25 μg of total RNA was separated by electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde, then transferred to nylon membranes. These were hybridized with probes labeled with digoxigenin, using a PCR DIG Probe Synthesis Kit. Hybridization and detection were performed according to

the methods described for our Southern blots.

RESULTS AND DISCUSSION

Cloning and Characterization of StGRXs

We performed *in silico* screening of ESTs to isolate potato cDNAs whose expression was induced 6 h after suberization. Three GRX clones -- StGRX1, StGRX2, and StGRX3 -- were identified from ESTs from a "6-hr post-suberization" cDNA library. Based on the abundance of each GRX among these potato ESTs, we could estimate the *in silico* level of expression and organ localization of each isoform. Out of a total of 1072 ESTs, 4 encoded GRX with a CxxC/S active site, 3 encoded GRX with a CCxC/S active site, and none encoded GRX with a CGFS active site. The CCxC/S type GRX was called StGRX1, and we named the two CxxC/S type GRXs StGRX2 and StGRX3 (Fig. 1). Based on the prediction software, TargetP (http://www.cbs.dtu.dk/services/TargetP/), we believe these are located in the cytosol. StGRX2 has an N-terminal extension, which supposedly directs proteins into yet-unspecified organelles. StGRX1, which encodes a 101-amino acid sequence, showed the greatest homology with glutaredoxin from *Arabidopsis thaliana* (71% identity, GenBank AAC63642) and *Oryza sativa* (58% identity, GenBank AAK53837). Hence, we identified the StGRX1 polypeptide as a potato homologue of glutaredoxin. The tetrapeptide CCxC, which constitutes the active site of glutaredoxin, was conserved within the potato StGRX1 sequence. StGRX2, which encodes a 125-amino acid sequence, showed the greatest homology with glutaredoxin from *Medicago truncatula* (72% identity, GenBank ABE93576), *Arabidopsis thaliana* (66% identity, GenBank BAB08846), and *Solanum lycopersicum* (64% identity, GenBank CAA77130), while StGRX3, encoding a 108-amino acid sequence, showed the greatest homology with glutaredoxin from *Solanum lycopersicum* (97.2% identity, GenBank Y18346).

Very few data are available concerning the distribution of GRXs in different plant organs, and even less is known about their intracellular localization. In potato tissues, e.g., leaves, stems, and roots, that were sampled during the vegetative-growth stage, StGRX1 transcripts accumulated to higher levels than in reproductive-stage tissues, such as the flower, stolon, and tuber. Transcripts of StGRX2 accumulated in an opposite pattern (Fig. 2). This expression study, therefore, suggests that the accumulation of glutaredoxin mRNAs is associated with growth stage. Northern blot analysis (Fig. 2) showed similar ethidium bromide staining in all samples, implying approximately equal loading of total RNA from the leaves, roots, stems, and flowers. To determine the copy number of these genes, we performed Southern blot analyses on genomic DNA extracted from leaf samples. Strong two- or three-band patterns were observed when three different restriction enzymes were used, thereby indicating that at least two copies of *StGRX1* and *StGRX3* are present in the potato genome. In comparison, *StGRX2* was associated with strong single- or double-band patterns, indicating single or double copies of that gene. Therefore, Southern blot analysis of genomic DNA suggests that the StGRX genes are members of a small gene family.

Expressions of StGRXs during Tuberization and Suberization

GRX is a glutathione (GSH)-dependent reductase, cata-

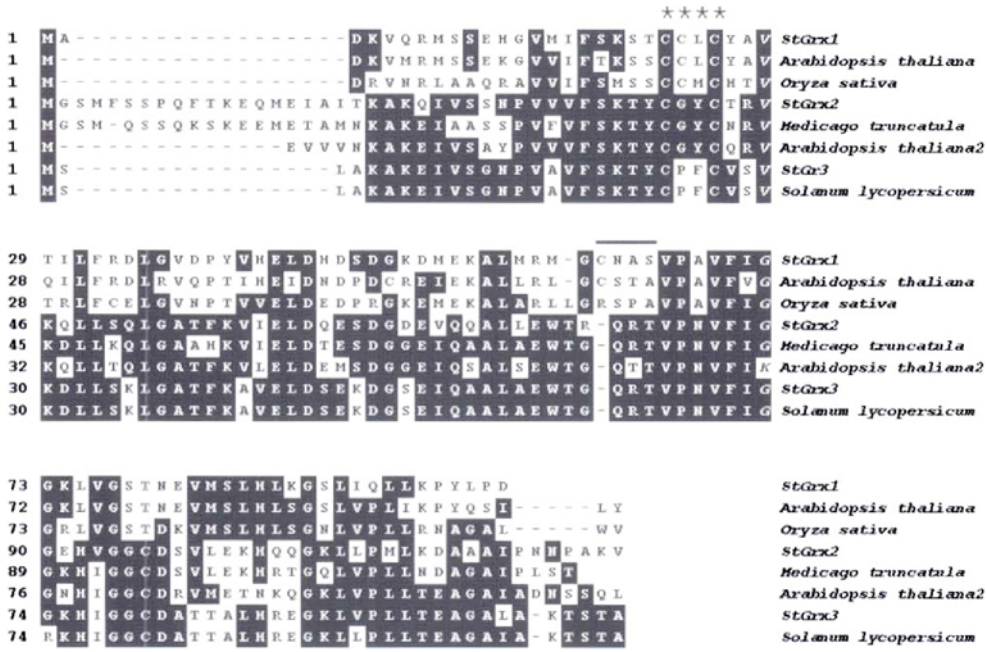

Figure 1. Alignment of predicted amino acid sequences from three StGRX genes of *Solanum tuberosum* L. cv. Desiree, using ClustalW program. Amino acid sequences conserved in more than two proteins are shaded; residues with asterisks correspond to conserved regions of dithiol CXXC active site; with dashed lines, correspond to monothiol CXXS motif. Compared sequences are *Arabidopsis thaliana* (GenBank AAC63642), *Oryza sativa* (GenBank AAK53837), *Medicago truncatula* (GenBank ABE93576), *Arabidopsis thaliana* (GenBank BAB08846), and *Solanum lycopersicum* (GenBank Y18346).

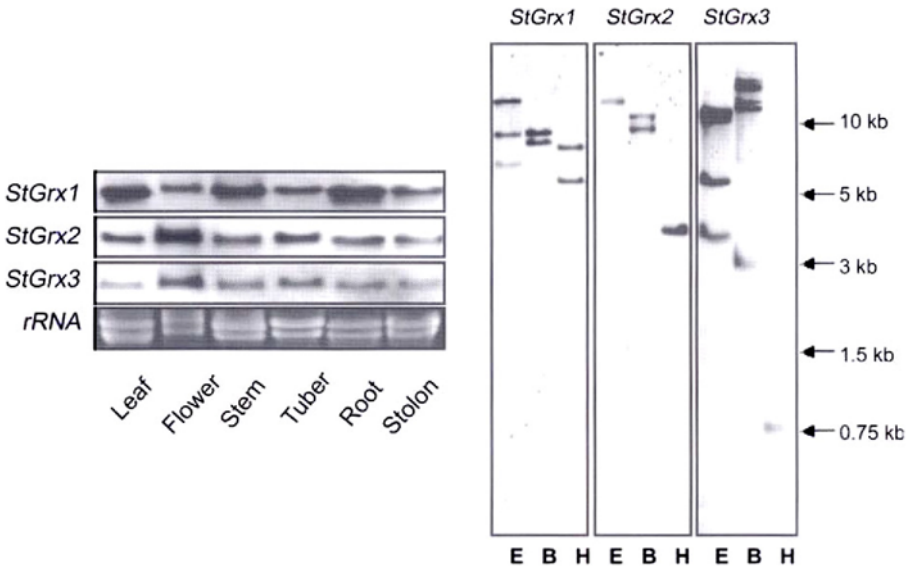

Figure 2. Copy number prediction of StGRX genes by genomic Southern blot analysis, and expression profiles in different tissues, based on northern blots. Genomic DNA was isolated and digested with appropriate restriction enzymes, then subjected to electrophoresis through 0.7% agarose gel before transfer to nylon membrane. Hybridization was performed with digoxigenin (DIG)-labeled probes. Target genomic DNA and RNAs were detected via chemiluminescence.

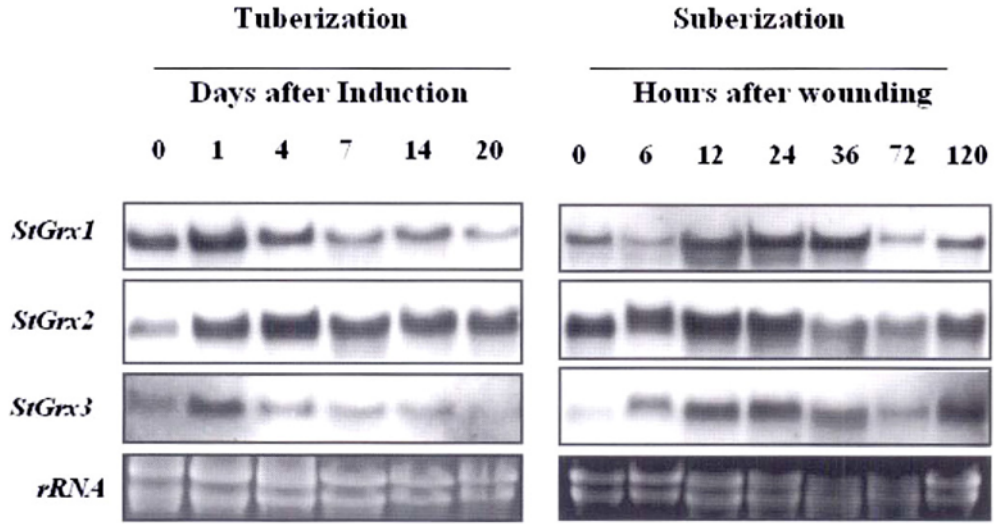

Figure 3. Expression profiles of three StGRX genes following northern blot analysis. Total RNA was isolated from nodal cuttings of potatoes after tuber induction, and from tubers during suberization after wounding at different time intervals. After separation on nylon membrane, total RNA was hybridized to probes of StGRX genes and was detected by chemiluminescence. Lower panels for each item show rRNA stained with EtBr.

lyzing the disulfide reduction of target proteins, such as ribonucleotide reductase. By altering the redox state of target proteins, this enzyme becomes involved in many cellular functions, including DNA synthesis, signal transduction, and defense against oxidative stress. Using StGRX1, StGRX2, and StGrx3 as probes, we examined the accumulation of glutaredoxin transcripts during the development of potato microtubers. Expression of StGRX1 mRNA decreased over time, whereas that of StGRX2 mRNA was maximally increased approximately 4 d after tuber induction and was maintained at that peak level during tuber maturation. Expression of StGRX3 was similar to, but weaker than, that of StGRX1 (Fig. 3). Previous tests showed that StGRX2 transcripts also accumulated in the reproductive tissues (Fig. 2). These results indicate that StGRX2 is induced in reproductive organs and is involved in their development. Because ROS is associated with tuberization in potatoes (Kim et al., 2007), StGRX2 might be related to redox regulation during tuberization via the modulation of hormone biosynthesis. For example, that gene may alter the redox state of specific proteins in the biosynthetic pathway.

The expression profiles of StGRXs were determined during suberization at 0, 6, 12, 24, 36, 72, and 120 h after wounding. That of StGRX1 mRNA decreased just after treatment was applied, then increased up to 36 h. Expression of StGRX2 mRNA increased until 24 h after wounding before decreasing during suberization. Transcript levels for StGRX3 were minimal before wounding, but then were

significantly induced afterward (Fig. 3). Our result suggests that this particular gene is involved in a response to wounding that is activated by either ethylene or MeJA. It is interesting that StGRX1 expression decreased just after wounding but then increased until 36 h post-suberization.

Oxidative damage and redox signaling can regulate the protein thio-redox state. A major means by which this occurs is through the response of protein thiols to changes in the glutathione (GSH) to glutathione disulfide (GSSG) ratio. The intracellular GSH/GSSG ratio usually is kept high (>99% reduced) through the reduction of GSSG to GSH by glutathione reductase, enabling GSH to act as an antioxidant (Jacob et al., 2003). Fluctuations in that ratio may cause protein thiols to change their redox state, enabling protein function to respond reversibly to redox signals or oxidative damage. However, during such events, ROS oxidizes GSH to GSSG directly; alternatively, oxidation of GSH may be catalyzed by glutathione peroxidases (Beer et al., 2004). Therefore, it seems that the decline we observed in StGRX1 expression just after wounding is associated with a rapid rise in ROS to defend against pathogen attack and promote healing during potato suberization.

Expression of StGRXs under Biotic and Abiotic Stresses

When plants are exposed to certain pathogens, cells in the immediate affected area undergo rapid cell death at and near the site of infection. This hypersensitive response (HR) limits the spread of such pests (Dangl and Jones, 2001). HR is characterized by the differential expression of various genes related to plant defense and programmed cell death. For example, the release of ROS (oxidative burst) is one of many rapid and transient changes observed during HR.

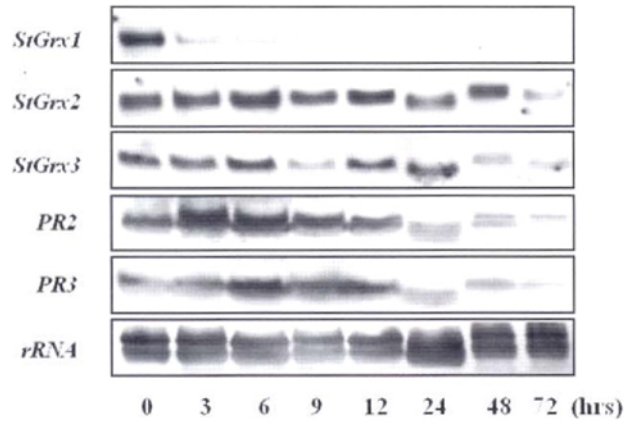

Figure 4. Expression of three StGRX and defense genes in potato after inoculation with *Phytophthora infestans*. Transcript levels of *PR-2*, *PR-3*, and *StGRX* in leaves of one-month-old plants were determined by RNA gel-blot analysis. Lower panels for each item show rRNA stained with EtBr.

Others include alterations in ion fluxes, protein phosphorylation patterns, pH, and membrane potential; as well as the oxidative cross-linking of plant cell wall proteins (Richberg et al., 1998). To investigate the roles of StGRXs in defense against fungal pathogens, we evaluated transcript levels of the PR-2, PR-3, and StGRX genes in leaves from one month-old potato plants inoculated with *Phytophthora infestans*. RNA gel-blot analysis showed that the expression of two defense marker genes was induced approximately 3 h after inoculation, then was decreased after 12 h (Fig. 4). Although no significant changes were found in the expression of StGRX2, that of the StGRX1 and -3 genes (the former, especially) was significantly reduced just after inoculation.

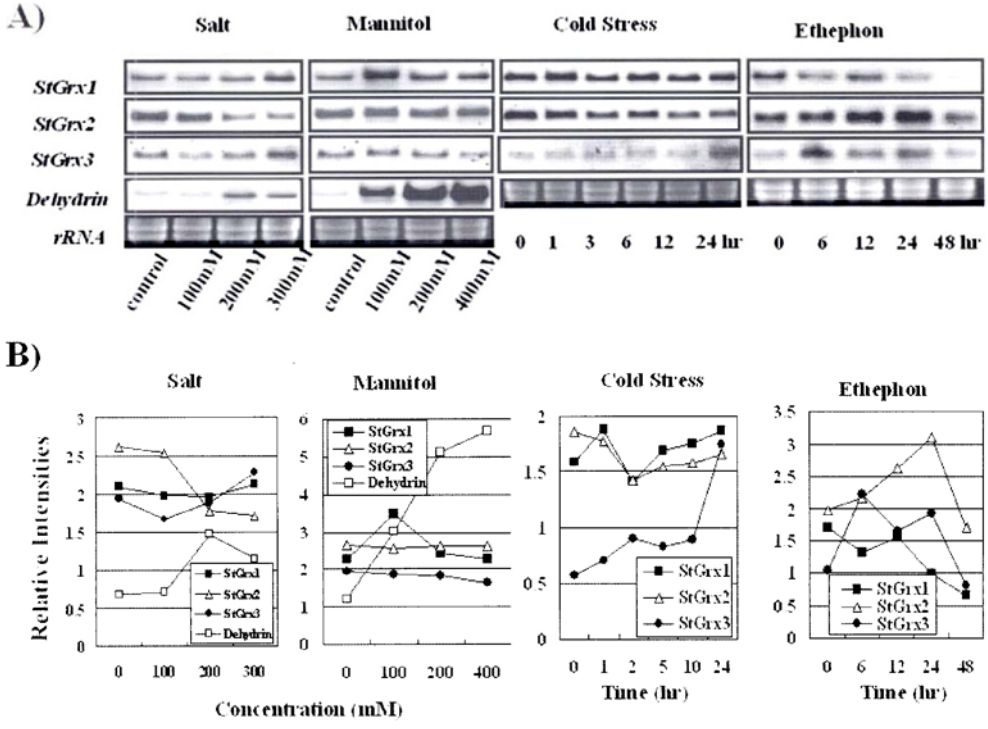

Figure 5. RNA gel blot analysis of three StGRX genes after treatment with: 0, 100, 200, or 300 mM NaCl; 0, 100, 200, or 400 mM mannitol; cold stress; or ethephon (**A**); and relative signal intensities (**B**). Lower panels for each item show rRNA stained with EtBr.

Thus, StGRX1 gene expression appears to be negatively associated with the defense response against fungal infection, and it is plausible that this action is strongly correlated with HR by facilitating the rapid accumulation of redox signals. In contrast, expression of StSRX2 and -3 did not immediately decline upon such exposure (Fig. 3), which strongly suggests that their physiological functions different from those of StGRX1.

When *in vitro* plants were treated with salt and mannitol, our RNA gel-blot analysis demonstrated that expression of the dehydrin gene, which was used as a marker gene, was induced by 24 h of treatment with 200 mM salt or 100 mM mannitol in vial cultures (Fig. 5). Although expression of the StGRX1 and StGRX3 genes was not significantly altered by either type of treatment, that of the StGRX2 gene was significantly reduced by treatment with 200 mM salt for 24 h, thus revealing a negative association between that gene and saline stress.

No significant changes were seen in the cold-induced expression of any of these three genes whereas, during ethephon treatment, that of the StGRX1 gene was reduced while StGRX2 expression was induced at 6 to 24 h after treatment. Expression of StGRX3 was slightly increased 6 h after such treatment before it gradually decreased (Fig. 5). These three glutaredoxins responded differently to treatment with plant hormones. Generally, the StGRX2 response was very sensitive. For example, after GA_3 or zeatin applications, induction of StGRX2 expression was greater than for the others; StGRX1 expression did not change significantly, and StGRX3 transcripts were only slightly elevated at 10 h post gibberellin treatment and at 1 h after MeJA exposure (Fig. 6).

Among our three putative potato GRX genes, the plant-specific CCxC/S type, StGRX1, is likely a housekeeping gene; its transcripts were observed in all organs and in response to various abiotic and biotic stresses, as well as to hormones. It also is likely that this gene is strongly correlated with HR and wounding by facilitating the rapid accumulation of redox signals during the defense against fungal pathogens and in the early stages of wounding. In contrast, the CxxC/S type, StGRX2, is induced upon exposure to various hormone treatments and in response to wounding, but is also constitutively expressed during pathogen attack and various abiotic stresses. Changes in StGRX3 transcript levels are quite similar to those of StGRX2, although the degree of that expression is rather low compared with the other two genes. Therefore, the differential expression of these three StGRXs strongly indicates their physiological functions are not the same.

Consistent with this hypothesis, at least two *Arabidopsis* GRXs have been genetically and functionally characterized. In that genome, at least 31 open-reading frames have been annotated to code putative GRXs. One CC-type GRX, *ROXY1*, is involved in floral petal development (Xing et al., 2005). Furthermore, the chloroplast-localized monothiol, AtGRXcp, with high similarity to yeast GRX5, protects plant cells against the oxidative damage of proteins (Cheng et al., 2006). Among our three StGRXs, only StGRX2 was predicted to contain a membrane-targeting domain (Fig. 1), but the prediction software used here -- TargetP -- could not specify into which organelle. This software assigned a score of 0.695 to "any other location", with the second highest score, 0.291, being "into the chloroplast". It is possible that StGRX2 may be putatively localized to the chloroplast, a major organelle that contributes to the production of

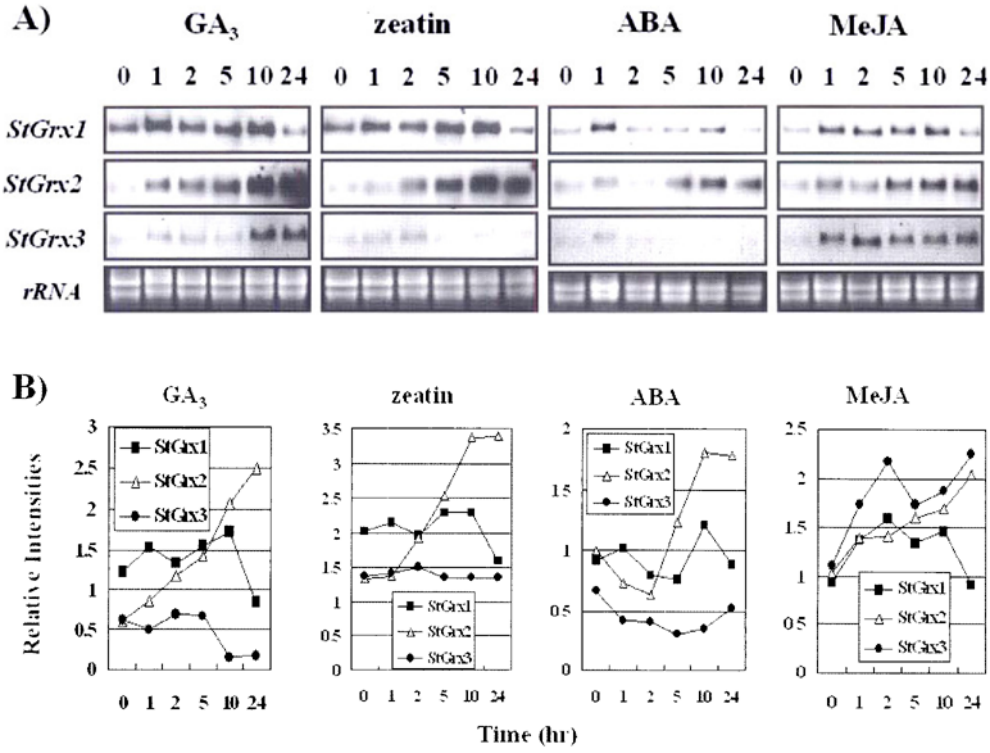

Figure 6. RNA gel blot analysis of three StGRX genes, with their respective probes, in response to treatments with GA_3, zeatin, ABA, or MeJA (**A**); and relative signal intensities (**B**). Lower panels for each item show rRNA stained with EtBr.

reactive oxygen species during photosynthesis (Rhoads et al., 2006). Here, StGRX2 transcript levels were up-regulated by treatments with gibberellin (GA_3) or cytokinin (zeatin), while StGRX1 was constitutively expressed, regardless of hormones being applied. We have recently proposed that ROS regulate potato stem growth and tuber development by modulating the GA biosynthetic pathway (Kim et al., 2007). If this is the case, StGRX2 likely plays a role in GA-mediated tuber development. Likewise, we have now determined that StGrx2 is highly up-regulated during *in vitro* tuberization (Fig. 2). Song et al. (2007) also have reported that ROS may function as signal molecules for auxin-induced ethylene production, mediating responses to various stimuli. In our current research, StGRX3 gene transcripts initially were less abundant than those of the StGRX1 and -2 genes, but were significantly up-regulated when plants were subjected to ethephon or MeJA treatments, or when suberization was induced by wounding. This result strongly suggests that such a response by StGRX3 is activated either by ethylene or MeJA alone or via synergism, as is evident during root-hair development (Zhu et al., 2006) as well as by defense responses against pathogens and herbivores (Lorenzo et al., 2003).

Very few data are available concerning the distribution and functioning of GRXs in different plant organs. Our results now provide a basic knowledge about the plausible roles for three potato glutaredoxins against various biotic and abiotic stresses. GRXs are able to reduce target proteins through dithio-disulfide exchanges that utilize the two active site cysteines in a manner similar to that of TRXs. Additionally, GRXs uniquely reduce mixed disulfides via a monothiol mechanism (Fernandes and Holmgren, 2004). By altering the redox state of target proteins, GRXs are involved in many cellular functions, including DNA synthesis, signal transduction, and defense against oxidative stress. Because plants appear to control various physiological processes by regulating the amount of ROS, its role as a signal transducer have been extensively studied. Those processes include pathogen defense (Levine et al., 1994; Alvarez et al., 1998), tolerance to abiotic stresses (Prasad et al., 1994), root development (Joo et al., 2001), senescence (Zimmermann and Zentgraf, 2005), stomatal behavior (McAinsh et al., 1996), and growth and tuberization (Kim et al., 2007). To regulate the level of ROS, the plant employs efficient redox and antioxidant systems, including the glutathione redox cycle, which is essential to the maintenance of the thio-redox state. Therefore, GRX expression is important to the fine-control of the redox state as a defense against biotic and abiotic stresses.

In conclusion, we have now demonstrated the differential expression of three potato StGRX genes, which suggests specific physiological roles for each during plant development and in response to various environmental cues. StGRX1 is a housekeeping gene, functioning in the rapid defense reaction to various abiotic and biotic stresses. In contrast, StGRX2 and -3 are selectively expressed during plant development and in response to internal and external signals. However, further studies with transgenic plants are necessary if we are to pinpoint the cellular localization and functioning of those StGRX genes.

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