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Proteomic analysis of Korean ginseng (*Panax ginseng* C. A. Meyer) following exposure to salt stress

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ABSTRACT We evaluated the response to salt stress of two different ginseng lines, STG3134 and STG3159, which are sensitive and tolerant, respectively, to salt treatment. Plants were exposed to a 5 dS/m salt solution, and chlorophyll fluorescence was measured. STG3134 ginseng was more sensitive than STG3159 to salt stress. To characterize the cellular response to salt stress in the two different lines, changes in protein expression were investigated using a proteomic approach. Total protein was extracted from detached salt-treated leaves of STG3134 and STG3159 ginseng, and then separated by two-dimensional polyacrylamide gel electrophoresis (2-DE). Approximately 468 protein spots were detected by 2-DE and Coommassie brilliant blue staining. Twenty-two proteins were found to be reproducibly up- or down-regulated in response to salt stress. Among these proteins, twelve were identified using MALDI-TOF MS and ESI-Q-TOF and classified into several functional groups: photosynthesis-related proteins (oxygen-evolving enhancer proteins 1 and 2, rubisco and rubisco activase), detoxification proteins (polyphenol oxidase) and defense proteins (β-1,3-glucanase, ribonuclease-like storage protein, and isoflavone reductase-like protein). The protein levels of ribonuclease-like storage protein, which was highly induced in STG3159 ginseng as compared to STG3134, correlated tightly with mRNA transcript levels, as assessed by reverse-transcription (RT)-PCR. Our results indicate that salinity induces changes in the expression levels of specific proteins in the leaves of ginseng plants. These changes may, in turn, play a role in plant adaptation to saline conditions.

Introduction

Ginseng (*Panax ginseng* C. A. Meyer) is commonly used as a tonic in traditional oriental medicine. However, the growth of ginseng is affected by a variety of environmental factors, including the water content of the soil, temperature, and light intensity. Recently, it was reported that ginseng is weakly tolerant to a range of soils affected by salt. Ginseng has been cultivated for a long period with heavy use of chemical fertilizers, which is one of the causes of salt accumulation in arable fields of ginseng. Thus, it is likely that ginseng is exposed to salinity stress during cultivation.

Salt stress severely limits plant growth (Banzai et al. 2002) and production (Amitai-Zeigersona et al. 1995). Plants suffer from multiple stresses due salinity, including water deficiency, ion toxicity and oxidative stress (Munns and Tester 2008). Resistance to salt stress requires alterations in the cellular

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machinery that are a direct result of changes of gene expression (Parker et al. 2006). Proteins that are induced by salt stress are involved in the accumulation of certain metabolites and cellular proteins (Kong-Negren et al. 2005). Studies of salinity tolerance in crop plants have ranged from genetic mapping to the molecular characterization of salt/drought-induced gene products (Quesada et al. 2002; Shinozaki and Yamaguchi-Shinozaki 2007). The identification of salt stress-responsive genes will require the application of recently developed molecular tools, such as genome-wide genetic and physical maps, microarray analysis, and proteomics.

Proteomics is the global analysis of proteins that are differentially expressed in a cell, tissue or organism at a given time or under certain conditions (Agrawal et al. 2006). Recently, proteomic approaches have been used to identify proteins in ginseng whose expression changes in response to high intensity light (Nam et al. 2003), in hairy roots (Kim et al. 2003), and in different parts of the ginseng plant and isolated ginseng plant cells (Lum et al. 2002). To date, cytosolic small heat-shock protein, cytosolic ascorbate peroxidase, putative major latex like protein, Rieske Fe/S protein, putative 3-β-hydroxysteroid dehydrogenase/isomerase-like protein, and oxygen-evolving enhancer-like protein have been identified as being either up- or down- regulated in response to light stress.

In the current study, we used a proteomic approach to analyze the molecular response to salt stress in ginseng leaves. Initially, we characterized selected salt-sensitive and salt-tolerant ginseng lines using a physiological marker of salt damage, chlorophyll fluorescence. To identify salt-responsive proteins, we compared the patterns of protein expression in salt-tolerant and salt-sensitive ginseng plants using 2-DE. To our knowledge, this is the first report of a time-resolved proteomic analysis of the salt response in ginseng.

Materials and methods

Plant materials and stress treatment

Local selection led to the release of the salt-sensitive (STG 3134) and salt-resistant (STG3159) ginseng lines that were used in this study. Salt tolerance is typically expressed in terms of the stage of plant growth over a range of electrical conductivity (EC) levels. A solution soil extract is prepared from the soil of interest, and an electrical current is applied to the solution in a glass cell using two electrodes. Units of conductivity are deciSiemens per meter (dS/m). The salt solution used in the current study was composed of 21 mM KNO_3, 6.1 mM KH_2PO_4 and 6.2 mM $MgSO_4 \cdot 7H_2O$ for a conductivity of 5 dS/m. Detached leaves of 4-year-old ginseng were placed in this salt solution for 7 days.

Protein extraction

Samples were extracted with Mg/NP-40 buffer [0.5 M Tris-HCl (pH 8.3), 2% (v/v) NP-40 (Amresco, USA), 20 mM $MgCl_2$, 2% (v/v) β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1% (w/v) polyvinylpolypyrrolidone (PVPP)], and then fractionated with polyethylene glycol (PEG) 4000, according to the method of Kim et al. (2004b). Briefly, ginseng leaves were placed in liquid nitrogen, transferred to a pre-chilled mortar, and ground to a fine powder with a pestle in liquid nitrogen. Samples were extracted with Mg/NP-40 buffer followed by centrifugation at 12000 x g for 15 min. Proteins in the supernatant were mixed with a 50% (w/v) PEG stock solution to a final concentration of 15% (w/v) PEG. The mixture was incubated on ice for 30 min and then subjected to centrifugation at 12000 x g for 15 min. The supernatant was collected, and then the proteins were precipitated with acetone. Protein concentration was measured according to the procedure of Lowry et al. (1951).

2-DE analysis

2-DE was performed according to the method of Kim et al. (2004a). For separation in the first dimension, we used isoelectric focusing (IEF). The gel mixture consisted of 4.5% (w/v) acrylamide, 9.5 M urea, 2% (v/v) NP-40, and 2.5% (v/v) pharmalytes (pH 3-10:pH 5-8:pH 4-6.5 at a ratio of 1:3.5:2.5 (Amersham Pharmacia Biotech, USA). Total protein extract (250 μg) was mixed with IEF sample buffer and then loaded onto an 18-cm IEF tube gel. IEF was performed at 600 V for 12 hours (h), 800 V for 6 h, and then 1000 V for 3 h. Following

IEF, the gel was placed into a 20 mL screw-cap tube with 5 mL of equilibration buffer containing 10% (v/v) glycerol, 2.5% (w/v) SDS, 125 mM Tris-HCl (pH 6.8), 5% (v/v) β-mercaptoethanol, and 0.1 mg/mL bromophenol blue. The gel was agitated gently at room temperature for 30 min, and then stained by colloidal Coommassie brilliant blue (CBB) G-250.

Image and data analysis

Images were acquired using a transmissive scanner (PowerLook III, UMAX) at 16-bit pixel depth, 300-dpi resolution, and the brightness and contrast set to default. Gel spots were automatically detected using ImageMaster 2D Platinum software 6.0 (GE Healthcare Bio-Sciences AB, Sweden). The intensity of each spot was normalized to the average spot intensity of the gel. The intensities of proteins that were differentially expressed in replicate gels were quantitatively measured to obtain statistical information on the variations in protein levels.

In-gel digestion

Colloidal CBB-stained proteins were excised from the gel, washed with 50% (v/v) acetonitrile in 0.1 M NH_4HCO_3, and then dried in a vacuum centrifuge (Hellman et al. 1995). Gel fragments were reduced in 20 μL of 10 mM DTT with 0.1 M NH_4HCO_3 for 45 min at 55℃. After cooling, the DTT solution was replaced with 55 mM iodoacetamide in 0.1 M NH_4HCO_3. After washing, the dried gel pieces were re-swollen in 10 μL digestion buffer containing 25 mM NH_4HCO_3 and 12.5 ng/mL trypsin (Promega, USA) and then incubated at 37℃ overnight. Tryptic peptides were extracted.

MALDI-TOF-MS

Samples were analyzed using a Voyager-DE STR MALDI-TOF-MS (PerSeptive Biosystems, USA), as follows. Parent ion masses were measured in the reflectron/delayed extraction mode with an accelerating voltage of 20 kV, a grid voltage of 76.000%, a guide wire voltage of 0.01%, and a delay time of 150 ns. A two-point internal standard for calibration was used with des-Arg1-Bradykinin (m/z 904.4681) and angiotensin 1 (m/z 1296.6853). Peptides were selected in the mass range of 700-3000 Da. For data processing, the MoverZ software program was used. Peak annotations were checked manually to prevent non-monoisotopic peak labeling. Monoisotopic peptide masses were used to search the databases, allowing a peptide mass accuracy of 50 ppm and one partial cleavage. To determine the confidence of the identification results, the following criteria were used: more than five peptides must be matched, and the sequence coverage must be greater than 15%. Database searches were performed using Protein Prospector (http://prospector.ucsf.edu), ProFound (http://www.unb.br/cbsp/paginiciais/profound.htm), and MASCOT (www.matrixscience.com).

Electrospray ionization-tandem mass spectrometry

Electrospray ionization-tandem mass spectrometry (ESI-MS/MS) was performed using a QSTAR pulsar-I MS system (Applied Biosystems/MDS Sciex, Canada) equipped with a nano-electropsray ion source (MDS Protana, Denmark), as described previously (Lee et al. 2007). Briefly, the ion-spray voltage was set to a potential of 900-1000 V and Scan data of the tryptic peptides was acquired over the m/z range 400-1200 Da in the positive mode. MS/MS mode was operated over the m/z range 80-2000 Da with manually optimized collision energy settings for each peptide. MASCOT was utilized for searching the NCBI database (http://www.ncbi.nlm.nih.gov) for identification and interpretation of the raw MS/MS data. The other parameters for searching were the digestion enzyme (trypsin), one missed cleavage, variable modifications of carbamidomethyl, peptide mass tolerance of 72 Da, fragment mass tolerance of 70.8 Da, and mass values monoisotopic.

RT-PCR

Total RNA was isolated from ginseng leaves that were exposed to salt stress, as described for protein extraction. First-strand cDNA was synthesized from 2 μg of total RNA (Lim et al. 2007). The cDNA was diluted 10-fold, and 1 μL of the diluted cDNA was used for PCR amplification with the following gene-specific primers: actin (accession no. 59938792), forward, 5'-GATGACATGGAAAAGATTTGGCATC-3'/reverse,

5'-TGTTGTACGACCACTAGCATACAGG-3'; ribonuclease-like storage protein (accession no. P83618), forward, 5'-ATGG GCAATGTTTGCCCTAAGGCTT- 3'/reverse, 5'-CCTTCCTG GTTTAGTAGCCCAACTA-3'; β-1,3-glucanase (accession no. 90309319), forward, 5'-AAACCCCGAACCTTCAATCCCTTTC-3'/reverse, 5'-GGAAGATTACCCTCATTGTGGCTA-3'. Amplified products were separated on a 1% agarose gel and photographed.

Results and Discussion

Chlorophyll fluorescence imaging of two ginseng lines, STG3134 and STG3159, in response to salt

The Korean ginseng lines STG 3134 and STG 3159 differ significantly in their salt tolerance. STG3134 is a salt sensitive line, whereas STG3159 is a tolerant line. Recently, it was shown that plants exhibit different functional levels of photosynthesis, depending on salinity levels (Moradi and Ismail 2007). Chlorophyll fluorescence imaging facilitates the selection of salt tolerant lines, as well as provides spatial information about the damage. To determine the level of salt damage in ginseng leaves, we measured chlorophyll fluorescence in STG3134 and STG3159 leaves exposed to salt stress conditions. As seen in Figure 1, red fluorescence, which indicates damage due to salt stress, accumulated to high levels in the leaves of the salt sensitive line STG3134 as compared to STG3159.

Proteomic analysis of two ginseng lines upon exposure to salt stress

To determine whether there were proteins that were specifically induced in STG3134 and STG3159 ginseng upon exposure to salt, total protein was extracted from detached salt-treated leaves and analyzed by 2-DE (Figure 2). Proteins were fractionated by centrifugation, and then the supernatant was precipitated with acetone. Resolubilized proteins were analyzed

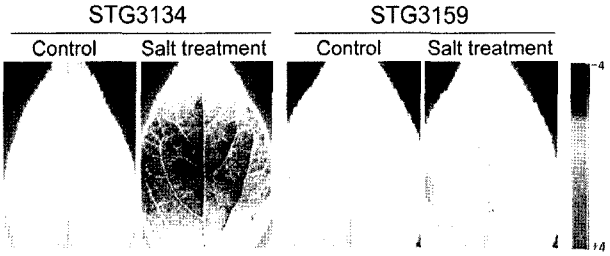

Figure 1. Chlorophyll fluorescence imaging of salt damage in the leaves of salt-sensitive (STG3134) and salt-resistant (STG 3159) ginseng. Red color indicates damage levels. The salt solution (5 dS/m) was composed of 21 mM KNO_3, 6.1 mM KH_2PO_4 and 6.2 mM $MgSO_4 \cdot 7H_2O$. Detached leaves of 4-year-old ginseng plants were placed in the salt solution for 7 days.

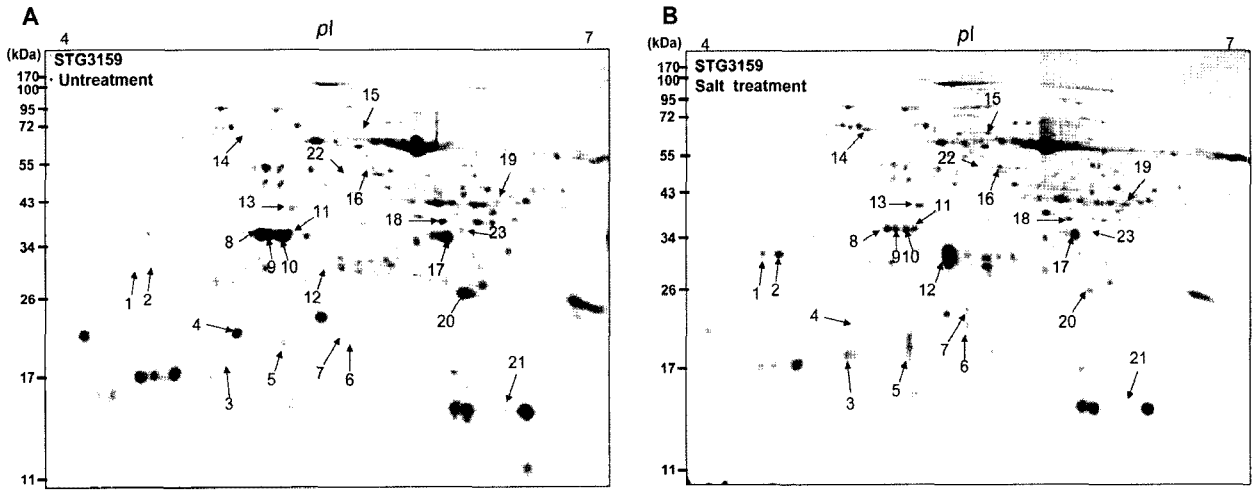

Figure 2. 2-DE analysis of PEG-fractionated proteins of STG3159 ginseng following salt stress. Detached leaves of 4-year-old plants were placed in a salt solution (5 dS/m) for 7 days and then harvested. Proteins (250 μg) were separated by 2-DE (p*I* 4-7) and then visualized by colloidal CBB staining. Twenty-three proteins that were either up- or down-regulated in response to salt are indicated by numbers. Relative molecular weight markers are indicated on the left in kDa. A, Control STG3159. B, Salt-treated STG3159

by 2-DE using IEF (pH 4-7) in the first dimension and SDS-PAGE (12.5%) in the second dimension. Representative 2-DE gels of proteins extracted from STG3159 leaves following exposure to salt are shown in Figure 2. Numerous protein spots were detected by CBB staining. The intensities of fifteen spots (spot nos. 1, 2, 3, 5, 6, 7, 12, 13, 14, 15, 16, 18, 19, 21, and 22) increased and eight (spot nos. 4, 8, 9, 10, 11, 17, 20, and 23) decreased in response to salt exposure. When we compared salt-tolerant and salt-sensitive ginseng, we found that spots 1, 3, 5, 6, 7, 12, 14, 15, and 16 were induced to significantly higher levels in salt-tolerant ginseng following exposure to salt, whereas the induction of spots 18, 21, and 22 was higher in the salt sensitive line (Figure 3). Figure 4 shows the results of the quantitative analysis of these differentially expressed proteins in STG3134 and STG3159 ginseng using ImageMaster software. These results indicated that the exposure of ginseng leaves to salt stress results in the differential expression of proteins in salt-tolerant and salt-sensitive plants.

Identification of differentially expressed proteins by MS

Several protein spots (as indicated in Figures 2 and 3) were excised from 2-DE gels and subjected to digestion using trypsin. Following extraction, the tryptic peptides were identified using MALDI-TOF-MS and ESI-Q-TOF. The results are listed in Tables 1 and 2. The proteins whose expression was induced in the leaves of ginseng in response to salt were identified as ribonuclease-like storage protein (spot no. 12), β-1,3-glucanase (spot 13), elongation factor Tu (EF-Tu, spot no. 16), isoflavone reductase-like protein (IFR, spot 18), and polyphenol oxidase (PPO, spot no. 19). Oxygen-evolving enhancer protein 1 (OEP1, spot nos. 8, 9, 10, and 11), ribulose bisphosphate carboxylase/oxygenase (RuBisCO, spot no. 17), RuBisCO activase (spot no. 18), and OEP2 (spot no. 20) were reduced in the leaves of ginseng treated with salt. We were unable to identify eleven spots (spot nos. 1, 2, 3, 4, 5, 6, 7, 14, 15, 21, and 22) by MS analysis due to the lack of a genome database for *Panax ginseng*.

Among the differentially expressed proteins, most were related to photosynthesis. We identified four spots (spot nos. 8, 9, 10, 11) that corresponded to OEP 1 of photosystem II (PS II), and one spot that corresponded to OEP 2 of PS II. The levels of these two proteins were markedly decreased by salt stress. OEP is involved in oxygen evolution and PS II stability (Sugihara et al. 2000). Severe stress conditions, such as exposure to ozone and sulfur dioxide, result in the suppression of

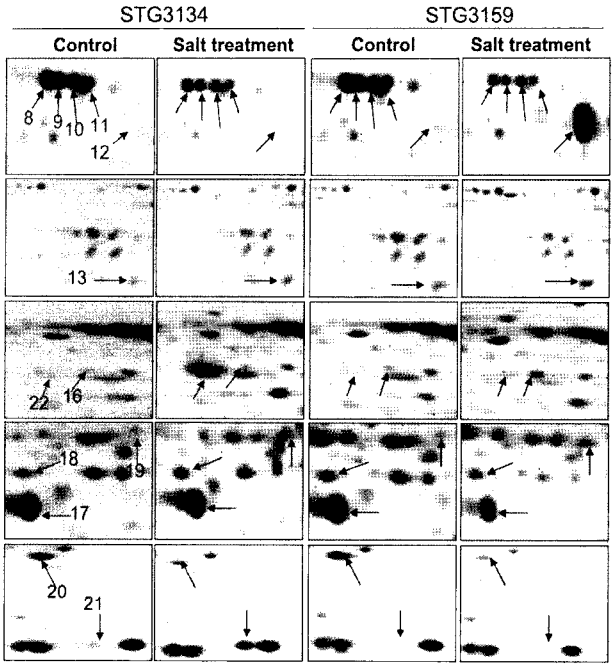

Figure 3. Magnified view of proteins that are differentially regulated by salt stress. Images are enlarged regions of 2-DE gels representing proteins that are differentially expressed in STG 3134 and STG3159 ginseng after treatment with salt (5 dS/m). Protein samples were analyzed as described for Figure 2.

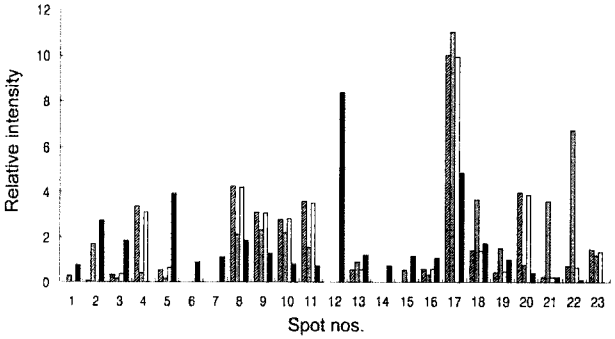

Figure 4. Quantitative analysis of proteins that were identified by 2-DE as differentially regulated by salt stress. Protein intensity was normalized, and data represents the mean expression levels from replicate samples. The quantification of 23 protein spots was carried out using ImageMaster 2D software. Bars represent the mean intensities of each spot. Marbled bars: control (untreated) STG3134; gray bars: salt-treated STG3134; white bars, control (untreated) STG3159; black bars, salt-treated STG3159.

Table 1. Identification of differentially induced proteins in response to salt stress using MALDI-TOF MS

Spot no.	MW/pI	Protein	Sequence coverage	Matched peptide	Accession no.	Source
8	35/5.6	Oxygen-evolving enhancer protein 1 (OEP1)	34%	8	P12359	*Spinacia oleracea*
9	35/5.6	Oxygen-evolving enhancer protein 1 (OEP1)	39%	8	P12359	*Spinacia oleracea*
10	35/5.6	Oxygen-evolving enhancer protein 1 (OEP)	41%	10	P12359	*Spinacia oleracea*
11	35/5.6	Oxygen-evolving enhancer protein 1 (OEP1)	39%	8	P12359	*Spinacia oleracea*
13	29/5.1	β-1,3-glucanase	31%	6	90309319	*Panax ginseng*
16	52/6.3	Elongation factor Tu (EF-Tu)	21%	8	P46280	*Glycine max*
17	51/5.7	Ribulose bisphosphate carboxylase/oxygenase (RuBisCO)	31%	19	57283378	*Lachnaea alpina*
22	48/5.5	RuBisCO activase	20%	7	13430332	*Zantedeschia aethiopica*

Table 2. Identification of differentially induced proteins in response to salt stress using ESI-Q-TOF

Spot no.	MW/pI	Amino acid sequences	Protein	Accession no.	Source
8	35/5.6	LTYTLDEIEGPFEVSSDGTVK DGIDYAAVTVQLPGGER	Oxygen-evolving enhancer protein 1 (OEP1)	P12359	*Spinacia oleracea*
10	35/5.6	LTYTLDEIEGPFEVSSDGTVK DGIDYAAVTVQLPGGER	Oxygen-evolving enhancer protein 1 (OEP1)	P12359	*Spinacia oleracea*
11	35/5.6	LTYTLDEIEGPFEVSSDGTVK DGIDYAAVTVQLPGGER	Oxygen-evolving enhancer protein 1 (OEP1)	P12359	*Spinacia oleracea*
12	27/5.9	FVDCPTDDATDDYR NENSEYVLTDINVCVNQQATR	Ribonuclease-like storage protein	P83618	*Panax ginseng*
16	52/6.3	ILDEALAGDNVGLLLR ETEKPFLMAIEDVFSITGR	Elongation factor Tu (EF-Tu)	P46280 836828	*Glycine max Chlorella sp. n1a*
18	34/6.3	FFPSEFGNDVDK	Isoflavone reductase-like protein (IFR)	19847822	*Cryptomeria japonica*
19	67/8.3	FYDENAQLVR	Polyphenol oxidase (PPO)	13559508	*Ananas comosus*
20	28/8.6	VDYLLGK	Oxygen-evolving enhancer protein 2 (OEP2)	P12302	*Spinacia oleracea*

OEP (Agrawal et al. 2002; Rakwal et al. 2003). Thus, our results were consistent with these previous observations. RuBisCO was also down-regulated in the leaves of ginseng in response to salt stress. RuBisCO proteins have been detected in drought-stressed sugar beet and metal-stressed rice leaves (Salekdeh et al. 2002; Hajheidari et al. 2005). These results suggest that photorespiration is affected by salt stress, and that the decrease in RuBisCO in response to salt stress reflects a decrease in photorespiration. In support of this hypothesis, RuBisCO activase, which activates RuBisCO (Law and Crafts-Brandner 2001), was markedly enhanced by salt stress (Figure 3). The accumulation of RuBisCO activase in response to other stresses, such as drought (Salekdeh et al. 2002), high temperature (Law and Crafts-Brandner 2001), and ozone (Agrawal et al. 2002) has also been reported. Thus, RuBisCO activase might be induced in response to salt stress, and activate RuBisCO. The expression of EF-Tu (spot no. 16) was induced more strongly in salt-tolerant STG3159 (Figure 3) ginseng as compared to STG3134. EF-Tu is a chloroplast protein. Recent studies have suggested that during maize chloroplast protein synthesis, EF-Tu plays a role in heat tolerance (Momcilovic and Ristic 2007) by functioning as a molecular chaperone (Rao et al. 2004). Recently, it was demonstrated that the chaperone activity of native pre-EF-Tu protects the photosynthetic enzyme rubisco activase from heat stress (Ristic et al. 2007). EF-Tu might function as a chaperone in other species as well, playing a role in the protection of house-keeping proteins, including those involved in photosynthesis, under conditions of salt or heat stress. Taken together, these results suggested that photosynthesis-related proteins are differentially regulated by environmental

stress.

We identified spot no. 18 in salt-treated ginseng leaves as IFR using ESI-Q-TOF (Table 2). The expression of IFR was much higher in STG3134 than in STG3159 ginseng. Several IFR genes are developmentally regulated or induced in response to biotic or abiotic stresses (Petrucco et al. 1996; Lers et al. 1998; Shoji et al. 2002; Kim et al. 2003). Recently, Kim et al. (2008) reported that rice IFR is involved in the cellular response to reactive oxygen species (ROS), based on proteomic analysis. The IFR family of proteins has been implicated in oxidative stress defenses because they contain a putative NAD(P)H binding domain, which is associated with oxidation/reduction properties (Babiychuk et al. 1995; Petrucco et al. 1996). We suggest that ginseng IFRs might act as detoxicants in response to salt stress. PPO (spot no. 19) was also induced by treatment with salt. PPO catalyzes the hydroxylation of monophenols to o-diphenols, and the subsequent oxidation of o-diphenols to o-diquinones. These compounds have been shown to be more effective antioxidants on a molar basis than vitamins C and E (Rice-Evans et al. 1997). While PPO is widely distributed in higher plants, the function of this protein *in vivo* is not yet clear. However, PPO has been suggested to play a role in plant resistance against diseases due to is high oxidizing capacity (Steffens et al. 1994; Hind et al. 1995; Trebst and Depka 1995, Li 2002). Recently, it was reported that PPO is induced in *Ramonda serbica* leaves by desiccation (Veljovic-Jovanovic et al. 2008). Thus, while it remains to be fully characterized, PPO may exhibit oxidizing activity against phenolic compounds that are released in ginseng following salt exposure.

As shown in Figure 4, ginseng ribonuclease-like storage protein (spot no. 12) was dramatically induced in STG3159 ginseng in response to salt treatment. This is the first report of ribonuclease-like storage protein function in the plant defense against salt stress. Recently, ribonuclease-like storage protein was found to be the most abundant protein in ginseng main root (Kim et al. 2004b). Furthermore, while it has high homology to plant RNase, ribonuclease-like storage protein does not possess RNase activity. Additional experiments using transgenic plants should help elucidate the role of this protein in salt-induced stress.

MALDI-TOF analysis revealed that spot 13 matched a β-1,3-glucanase with a coverage of 31% (Table 1), and we identified this protein as acidic β-1,3-glucanase (MW, 29 kD, pI 5.1). The β-1,3-glucanases have been reported to be induced in many plants following infection by various pathogens, as well as during development and in response to abiotic stresses, such as cold, drought, and salt (Hwang et al. 2007). Our results suggested that ginseng β-1,3-glucanase might also be involved in the plant response to salt stress. To determine whether the mRNA expression levels of ribonuclease-like storage protein and β-1,3-glucanase correlated with the changes in protein levels that we observed by 2-DE, we analyzed total RNA isolated from salt-treated ginseng leaves by RT-PCR (Figure 5). The mRNA expression levels of ribonuclease-like storage gene correlated well with the protein expression levels (spot no. 12) observed by 2-D. However, the mRNA levels of β-1,3-glucanase (spot no. 13), which exhibited increased protein levels in response to salt, were reduced, which indicated that the kinetics of protein expression β-1,3-glucanase differ from that of mRNA transcript accumulation.

In summary, we have carried out an analysis of the protein expression profiles of two different ginseng plant lines, STG 3134 and STG3159, in response to salt stress. Chlorophyll fluorescence imaging revealed that STG3159 was tolerant to salt stress, while STG3134 was salt-sensitive. These results were in good agreement with the phenotypes of these two lines

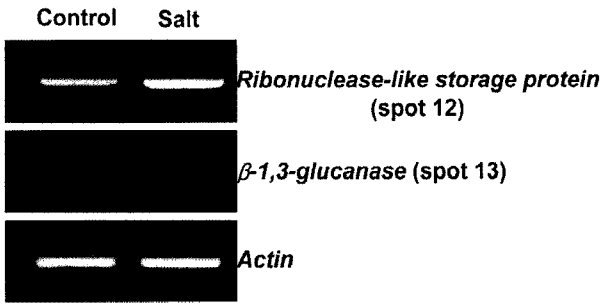

Figure 5. Verification of the protein expression levels of ribonuclease-like storage protein and β-1,3-glucanase genes using RT-PCR. Total RNA was isolated from ginseng leaves (STG 3159) exposed to salt stress (5 dS/m) for 7 days. Amplified PCR products were separated on a 1% agarose gel containing ethidium bromide, and then photographed. Actin was used as an internal control to normalize for cDNA concentration in each sample.

in field tests. Proteomic analysis using 2-DE coupled with MS led to the identification of proteins that might be involved in the adaptation of ginseng to salt stress. Variable levels in fourteen protein spots were revealed by 2-DE analysis of plants exposed to salt stress, leading to the identification of proteins associated with photosynthesis (OEP 1 and 2, RuBisCO, and RuBisCO activase), detoxification (PPO), and defense (β-1,3-glucanase, ribonuclease-like storage protein, and IFR). Of these proteins, β-1,3-glucanase and ribonuclease-like storage protein were more highly induced in STG3159 than STG3134, which indicates that they might play a key role in salt tolerance. In addition, the protein levels of ribonuclease-like storage protein, which was highly induced in STG3159 plants in response to salt, correlated tightly with mRNA transcript levels. The identification of salt-induced proteins by 2-DE will increase our understanding of the salt response mechanism in ginseng, and might lead to applications that aid in the breeding of plants for enhanced salt tolerance.

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