Comparative Genomics in Salt Tolerance between Arabidopsis and Arabidopsis-Related Halophyte Salt Cress Using Arabidopsis Microarray[1]

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Salt cress (*Thellungiella halophila*), a halophyte, is a genetic model system with a small plant size, short life cycle, copious seed production, small genome size, and an efficient transformation. Its genes have a high sequence identity (90%–95% at cDNA level) to genes of its close relative, Arabidopsis. These qualities are advantageous not only in genetics but also in genomics, such as gene expression profiling using Arabidopsis cDNA microarrays. Although salt cress plants are salt tolerant and can grow in 500 mM NaCl medium, they do not have salt glands or other morphological alterations either before or after salt adaptation. This suggests that the salt tolerance in salt cress results from mechanisms that are similar to those operating in glycophytes. To elucidate the differences in the regulation of salt tolerance between salt cress and Arabidopsis, we analyzed the gene expression profiles in salt cress by using a full-length Arabidopsis cDNA microarray. In salt cress, only a few genes were induced by 250 mM NaCl stress in contrast to Arabidopsis. Notably a large number of known abiotic- and biotic-stress inducible genes, including *Fe-SOD*, *P5CS*, *PDF1.2*, *AtNCED*, P-protein, β-glucosidase, and *SOS1*, were expressed in salt cress at high levels even in the absence of stress. Under normal growing conditions, salt cress accumulated Pro at much higher levels than did Arabidopsis, and this corresponded to a higher expression of *AtP5CS* in salt cress, a key enzyme of Pro biosynthesis. Furthermore, salt cress was more tolerant to oxidative stress than Arabidopsis. Stress tolerance of salt cress may be due to constitutive overexpression of many genes that function in stress tolerance and that are stress inducible in Arabidopsis.

Abiotic stresses greatly affect plant growth and crop production. To survive against these stresses, plants respond and adapt with complex mechanisms, including developmental, morphological, physiological, and biochemical strategies. High salinity is a major abiotic stress causing both osmotic and ionic stress. The adaptive strategy to osmotic stress is the accumulation of osmoprotectants such as Pro, glycinebetaine, mannitol, and the raffinose family oligosaccharides. This strategy has been shown to be important in improving stress tolerance in plants by manipulating genes encoding key enzymes of the osmoprotectant synthesis or degradation pathway (Tarczynski et al., 1993; Kavi-Kishor et al., 1995; Thomas et al., 1995; Nanjo et al., 1999a, 1999b; Taji et al., 2002). A high level of Na^+ is toxic to plants because it disturbs cytoplasmic K^+/Na^+ homeostasis. To prevent Na^+ accumulation in the cytoplasm, plants use the following three strategies: reducing Na^+ influx, vascuolar compartmentation of Na^+, and excretion of Na^+ via plasma membrane Na^+/H^+ antiporters (Ward et al., 2003). Overexpression of the vacuolar Na^+/H^+ antiporter *NHX1* has been shown to confer salt tolerance in Arabidopsis and tomato, suggesting the utility of this vascuolar compartmentation of Na^+ (Apse et al., 1999; Zhang and Blumwald, 2001). Also, overexpression of a plasma membrane Na^+/H^+ antiporter gene in a freshwater cyanobacterium conferred extreme salt tolerance and allowed growth in seawater, indicating the effectiveness of excreting Na^+ via plasma membrane Na^+/H^+ antiporters for salt tolerance (Waditee et al., 2002). Furthermore, very recently, overexpression of *SOS1*, a plasma membrane Na^+/H^+ antiporter, was shown to confer salt tolerance by

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retrieving Na^+ from the xylem of transgenic plants (Shi et al., 2003).

Although there have been many reports on salt tolerance, most of the studies have been on a typical glycophyte, Arabidopsis, and not on halophytes. This is because the technological advantages of this model plant are too significant, and halophytic plants have not been shown to be a suitable genetic and genomic model system. Recently, the halophytic plant species salt cress (*Thellungiella halophila*) was reported to grow in high salinity coastal areas in eastern China. Salt cress is thought to be a good model plant for the analysis of high salinity tolerance because it is closely related to Arabidopsis and has good genetic features such as similar morphology, small size, short life cycle, high seed number, and the ability to self-pollinate. Furthermore, salt cress has a gene composition with >90% nucleotide identity with that of Arabidopsis, and it can be transformed in planta according to the Arabidopsis protocol (Bressan et al., 2001; Zhu, 2001).

Although Arabidopsis is a typical glycophyte that is not particularly salt tolerant, a number of recent studies suggest that it may contain most, if not all, of the salt tolerance genes that one might find in halophytes (Shinozaki and Yamaguchi-Shinozaki, 2000; Zhu, 2000; Shinozaki et al., 2003). It is now hypothesized that a substantial percentage of halophytes use the mechanisms of salt tolerance found in glycophytes and that subtle differences in regulation result in large variations in tolerance or sensitivity between glycophytes and halophytes. This hypothesis can be tested by applying comparative genomics using microarray for genome-scale gene expression. Fortunately, since expressed sequence tag (EST) analyses of several hundred salt cress clones revealed 90% to 95% identities between salt cress and Arabidopsis cDNA sequences (Bressan et al., 2001), we assumed that a full-length cDNA microarray composed of 7,000 Arabidopsis genes could be applied in this case (Seki et al., 2002b). Recently, a microarray containing 8,987 cDNAs of rice EST was used to analyze the gene expression profile in barley roots during Fe-deficiency stress (Negishi et al., 2002). Genes of cereal crops tend to be highly conserved at the DNA sequence level (Devos and Gale, 2000), and this conservation allows the use of heterologous probes to identify orthologous DNA sequences among different species in DNA hybridization experiments.

In this study, we applied the full-length Arabidopsis cDNA microarray to reveal the differences in the regulation of salt tolerance mechanisms between a glycophyte, Arabidopsis, and a halophyte, salt cress. Furthermore, we compared the expression profiling data of salt cress with our previous Arabidopsis expression profiling data of genes that included not only those obtained by various abiotic stress treatments but also those obtained by biotic stress-related treatments using the same full-length Arabidopsis cDNA microarray. Salt stress tolerance of salt cress is discussed by comparison of Arabidopsis based on gene expression profiling of stress-inducible genes.

RESULTS

Salt Cress Is Extremely Salt Tolerant

The germination rate of salt cress in the absence of 4°C stratification was very low and not uniform. Stratification treatment at 4°C for 1 week greatly improved the low germination rate. Growth of the salt cress seedling was slower than that of Arabidopsis. Thus, it was necessary to examine the salt stress tolerance of salt cress and Arabidopsis either with seedlings of the same size or at the same age. Three-week-old Arabidopsis and 4-week-old salt cress plants were exposed to 500 mM NaCl solution to investigate the salt tolerance with seedlings of the same size. The salt tolerance of seedlings at the same age was examined using 3-week-old Arabidopsis and the salt cress plants exposed to 500 mM NaCl solution. After 3 weeks of treatment, complete chlorosis was observed in all the Arabidopsis plants of the same size and same age. By comparison, the salt cress plants were not affected by either condition (Fig. 1, A and B).

Salt Tolerance in the Hydroponic Culture System and Differences in NaCl Uptake

The demonstration of extreme salt tolerance of salt cress plants implies the existence of important mechanisms for salt tolerance in the root. It was necessary to gather not only the rosette leaves but also the roots. However, we could not harvest the root of the soil-grown Arabidopsis and salt cress plants. So we adopted the hydroponic culture system with glass beads (Nanjo et al., 1999a). This method enabled us to collect the roots easily and to change the salinity condition of the growth media reversibly. To check the salt tolerance on the system, we exposed 3-week-old Arabidopsis and 4-week-old salt cress plants of the same size to 250 mM NaCl solution (Fig. 2A). After a 24-h treatment, salt cress plants were not affected by the salinity stress, whereas significant chlorosis was observed in Arabidopsis. After a 96-h treatment, Arabidopsis plants showed complete chlorosis, conversely one-half of the salt cress plants survived. Consequently, we also confirmed the salt tolerance of salt cress in the hydroponic system.

To investigate the importance of salt cress roots in the tolerance to salt stress, we measured the accumulation of NaCl in the leaves of Arabidopsis and salt cress plants during high salinity stress in the hydroponic culture system. Three-week-old Arabidopsis and 4-week-old salt cress plants were exposed to 250 mM NaCl solution for 2, 5, 10, and 24 h. After exposure for 10 or 24 h, NaCl uptake of salt cress plants became slower than that of Arabidopsis (Fig. 2B). This suggested that salt cress roots possess special mechanisms to prevent inflow of NaCl.

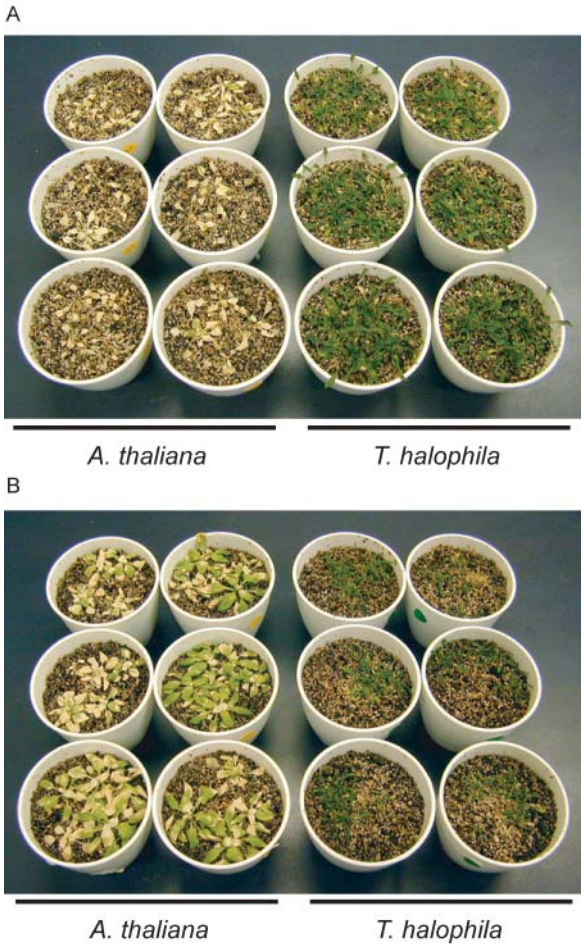

Figure 1. Survivability of salt cress and Arabidopsis under high-salt stress. A, High-salt stress tolerance of salt cress and Arabidopsis of the same size. Three-week-old Arabidopsis and 4-week-old salt cress plants that were the same in size were exposed to 500 mM NaCl solution for 3 weeks. B, High salt stress tolerance of salt cress and Arabidopsis of the same age. Three-week-old Arabidopsis and salt cress plants that were the same in size were exposed to 500 mM NaCl solution for 3 weeks.

Salt Stress-Inducible Genes in Salt Cress

To analyze the molecular mechanisms of salt tolerance in salt cress, we compared gene expression profiles in salt cress with those of Arabidopsis by using a full-length cDNA microarray (approximately 7,000 Arabidopsis genes). The Arabidopsis full-length cDNA microarray is expected to hybridize mRNA of salt cress more efficiently than Arabidopsis oligonucleotide microarray because the oligonucleotide microarray needs strict identity between mRNAs in comparison with the full-length cDNA microarray. In addition, since salt cress genes share >90% nucleotide identity with Arabidopsis genes, efficient cross-hybridization was expected.

To identify salt stress-inducible genes in salt cress and Arabidopsis, we treated plants with salt at 250 mM NaCl for 2 h. The reasons for using this condition are as follows: (1) genes rapidly inducible should be important for salt tolerance; (2) significant chlorosis is not observed within 2 h in control Arabidopsis; and (3) NaCl is taken up more efficiently at 10 h of salt treatment in Arabidopsis. The salt stress-inducible genes in salt cress and Arabidopsis were identified by microarray analyses that hybridized with Cy3 and Cy5 fluorescence-labeled probe pairs of salt-stressed plants plus unstressed plants. Subsequently, we investigated the differences in salt-inducible genes between salt cress and Arabidopsis by comparing their microarray data. Down-regulated genes could not be evaluated precisely because of lower hybridization signals. These genes might include not only down-regulated genes but also ortholog genes with low homology. Therefore, we concluded that the expression data obtained with heterologous microarray analysis can be used for the identification of up-regulation of genes.

Tables I and II show the genes that are up-regulated (ratio = $\log_2$ Cy5/Cy3 $\geq$ 1.5) by salt stress with a signal value greater than 3,000 in salt cress and Arabidopsis, respectively. We identified only 6 salt stress-inducible genes whose $\log_2$ ratios of salt stress versus nonstress were $\geq$1.5 in salt cress, whereas we showed 40 such salt stress-inducible genes in Arabidopsis. Figure 3 shows the classification of salt stress-inducible genes in salt cress and Arabidopsis. These genes were categorized into three groups: (1) genes induced in Arabidopsis; (2) genes induced in salt cress; and (3)

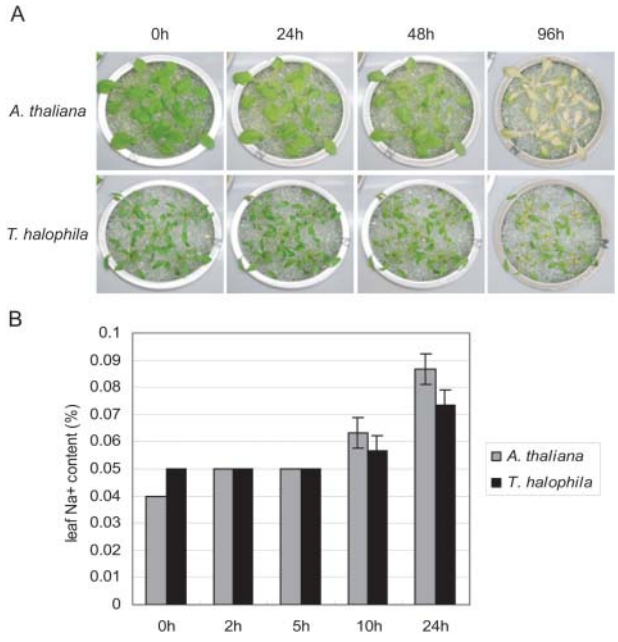

Figure 2. Evaluation of salt tolerance and Na^+ uptake in hydroponic culture system. A, High salinity stress tolerance in hydroponic culture system. Three-week-old Arabidopsis and 4-week-old salt cress plants that were the same in size were exposed to 250 mM NaCl solution for 24, 48, and 96 h. B, Accumulation of NaCl in leaves of Arabidopsis and salt cress during high salt stress in hydroponic culture system. Three-week-old Arabidopsis and 4-week-old salt cress plants that were the same size were exposed to 250 mM NaCl solution for 2, 5, 10, and 24 h.

Table I. *Genes up-regulated in salt cress under 250 mM NaCl stress for 2h*

Clone Name[a]	MIPS[b]	Gene[c]	Log_2 Ratio[d]	SD[e]
RAFL09-15-K07	At4g39800	Myoinositol-1-phosphate synthase	2.48	0.13
RAFL09-14-B02	At2g22240	Putative myoinositol 1-phosphate synthase	2.09	0.09
RAFL08-08-L20	At1g56600	Galactinol synthase, AtGolS2	1.72	0.32
RAFL08-16-M12	At2g33380	Putative calcium-binding EF-hand protein, responsive to desiccation, RD20	1.68	0.65
RAFL08-11-C23	At5g06760	Late embryogenesis abundant protein LEA-like	1.67	0.15
RAFL05-13-C23	At5g53450	Protein kinase family	1.51	0.07

[a]RIKEN Arabidopsis full-length (RAFL) cDNA. [b]MIPS protein code. [c]Indicates the putative functions of the gene products that are expected on the basis of sequence similarity. [d]Ratio = $\log_2$ Cy5/Cy3. [e]Standard deviation.

genes induced in both Arabidopsis and salt cress. The genes induced by salt stress in salt cress include myoinositol-1-phosphate synthase (INPS) gene. In the halophyte common ice plant (*Mesembryanthemum crystallinum*), known to accumulate methyl inositol, D-ononitol, and D-pinitol, which serves as an osmoprotectant, *INPS* RNA levels are up-regulated and levels of free myoinositol accumulate during salinity stress (Ishitani et al., 1996). By contrast, Arabidopsis does not show up-regulation of *INPS* or increased levels of myoinositol under salinity stress (Ishitani et al., 1996). Thus, salt cress may accumulate myoinositol, D-ononitol, and D-pinitol during salt stress as in the ice plant. Furthermore, galactinol synthase gene *AtGolS2* is up-regulated in salt cress under salt stress. Galactinol synthase catalyzes the first step in the biosynthesis of the raffinose family oligosaccharides. The transgenic plants that overaccumulate galactinol and raffinose have improved tolerance to drought or freezing (Taji et al., 2002; Pennycooke et al., 2003). Myoinositol is also used as a substrate of raffinose. Consequently, salt cress may also accumulate more raffinose and other raffinose family oligosaccharides than Arabidopsis during salt stress.

Genes Up-Regulated in Salt Cress Compared with Arabidopsis under Normal Growth Conditions

To compare expression profiles under normal growth conditions between salt cress and Arabidopsis, we hybridized the full-length Arabidopsis cDNA microarrays with Cy3 and Cy5 fluorescence-labeled probe pairs of nonstressed salt cress plus nonstressed Arabidopsis plants. Interestingly, a number of abiotic or biotic stress-inducible genes were expressed under normal growth conditions in salt cress (Table III; Fig. 4). Especially, the tendency observed in the genes up-regulated to high levels, and five of the eight genes with $\log_2$ ratios greater than 2.5 are known to be important genes in abiotic stress or biotic stress tolerance. The up-regulated genes are Fe-superoxide dismutase (*SOD*); 9-cis-epoxycarotenoid dioxygenase (*AtNCED2*); chitinase; plant defensin1.2 (*PDF1.2*); Δ-1-pyrroline-5-carboxylate synthetase (*AtP5CS*); plasma membrane Na^+/H^+ antiporter (*SOS1*); P-protein associated with nitric oxide (NO) production; and β-glucosidase.

We have performed many kinds of microarray analyses to analyze gene expression profiles in response to various abiotic, biotic stress, and oxidative stress in Arabidopsis using this full-length Arabidopsis cDNA microarray (Seki et al., 2002a, 2002b; Narusaka et al., 2003). Using the database of those microarray analyses, we analyzed the expression of the genes in Table III that were highly expressed (ratio; $\geq$1.5) in salt cress under normal growth conditions with Arabidopsis genes induced by various abiotic, biotic, and oxidative stress treatments. The abiotic stresses include drought, salt (NaCl), cold, and abscisic acid (ABA) treatment. The biotic and oxidative stress treatments include inoculation with a fungal pathogen, *Alternaria brassicicola*, to wild-type Arabidopsis (Columbia) and *pad3-1* mutant (Glazebrook and Ausubel, 1994), signal molecules (salicylic acid [SA], jasmonic acid [JA], and ethylene [ET]), reactive oxygen species-inducing compounds (paraquat and rose Bengal), and UV-C treatment, which causes cell death (Narusaka et al., 2003). Figure 4 shows the results of cluster analysis of the resultant expression profiles. The expression profiles include five groups, i.e. abiotic stress-inducible genes, biotic stress-inducible genes, both abiotic and biotic inducible genes, abiotic and biotic noninducible genes, and abiotic and biotic suppressible genes (Table III). Interestingly, one-half of the genes belonged to the groups that were abiotic, biotic, or both abiotic and biotic stress-inducible genes in Arabidopsis (Fig. 4, A and B). The fact that many stress-inducible genes were expressed under normal growth conditions in salt

Table II. *Genes up-regulated in Arabidopsis under 250 mM NaCl stress for 2h*

Clone Name[a]	MIPS[b]	Gene[c]	Log_2 Ratio[d]	SD[e]
RAFL07-11-M21	At5g52310	Hydrophilic protein, responsive to desiccation, RD29A	3.77	0.09
RAFL08-16-M12	At2g33380	Putative calcium-binding EF-hand protein, responsive to desiccation, RD20	3.52	0.15
RAFL05-21-C17	At4g27410	NAM-like protein and ATAF-like protein, responsive to desiccation, RD26	2.28	0.39
RAFL04-12-G16	At2g43570	Putative endochitinase	2.25	0.25
RAFL05-12-M18	At4g09010	Ascorbate peroxidase, putative	2.13	0.1
RAFL08-12-H04	At2g26690	Putative nitrate transporter	2.12	0.39
RAFL05-20-O23	At2g39800	Putative Δ-1-pyrroline 5-carboxylase synthetase, AtP5CS1	2	0.29
RAFL05-10-D11	At1g73480	Lysophospholipase homolog, putative	1.96	0.15
RAFL08-11-P07	At5g17460	Unknown protein	1.95	0.14
RAFL09-66-C10	At3g57260	β-1,3-glucanase 2, BG2	1.93	0.07
RAFL05-21-F13	At1g16850	Unknown protein	1.9	0.42
RAFL05-21-C06	At2g24850	Putative tyrosine aminotransferase	1.89	0.39
RAFL05-05-G20	At3g02480	Unknown protein	1.86	0.36
RAFL05-14-E16	At1g62570	Similar to glutamate synthase	1.84	0.23
RAFL05-08-P17	At1g20450	Group II LEA protein, early responsive to desiccation, ERD10	1.84	0.08
RAFL07-15-N09	At4g04020	Plastid-lipid associated protein PAP1	1.76	0.26
RAFL06-09-E13	ND[f]	Unknown protein	1.76	0.14
RAFL05-10-J09	At1g78070	Unknown protein	1.73	0.03
RAFL05-11-A18	At4g37590	Phototropic response protein family	1.69	0.27
RAFL05-16-I09	At5g20830	Suc-UDP glucosyltransferase	1.67	0.3
RAFL05-16-H23	At4g28140	AP2 domain transcription factor, putative	1.66	0.24
RAFL09-09-I16	At5g13630	Cobalamin biosynthesis protein	1.65	0.04
RAFL08-08-L20	At1g56600	Galactinol synthase, AtGolS2	1.63	0.81
RAFL05-14-A12	ND	Putative myosin heavy chain	1.62	0.17
RAFL08-11-H16	At3g14440	9-cis-epoxycarotenoid dioxygenase, AtNCED3	1.62	0.34
RAFL08-09-C23	At1g54100	Aldehyde dehydrogenase, putative	1.6	0.05
RAFL05-17-I08	At2g43020	Putative amine oxidase	1.59	0.19
RAFL07-07-G15	At1g01720	NAC domain protein, putative	1.59	0.82
RAFL04-16-P21	At4g37370	Cytochrome P450-like protein	1.58	0.41
RAFL05-11-I09	At5g52300	Hydrophilic protein, responsive to desiccation, RD29B	1.58	0.66
RAFL05-21-L12	At3g22830	Heat shock transcription factor-like protein	1.57	0.53
RAFL07-07-J02	At4g24960	ABA-induced like protein, AtHVA22d	1.57	0.43
RAFL05-19-I05	At1g01720	Strong similarity to OsNAC6 protein from *Oryza sativa*	1.56	0.27
RAFL06-07-B19	At3g11410	Protein phosphatase 2C, PP2C	1.55	0.18
RAFL05-19-O22	ND	Unknown protein	1.54	0.83
RAFL09-06-L09	At3g12580	Heat shock protein 70	1.52	0.16
RAFL05-03-A05	At2g42540	Cold-regulated protein, cor15a	1.51	0.3
RAFL05-15-H13	At5g25610	Responsive to desiccation, RD22	1.51	0.26
RAFL05-14-J01	At2g29450	Glutathione *S*-transferase	1.51	0.26
RAFL06-12-H12	At5g02020	Unknown protein	1.51	0.26
RAFL05-15-A16	At5g53120	Spermidine synthase	1.5	0.26
RAFL05-15-E19	At3g05640	Putative protein phosphatase 2C, PP2C	1.5	0.09

[a]RIKEN Arabidopsis full-length (RAFL) cDNA. [b]MIPS protein code. [c]Indicates the putative functions of the gene products that are expected on the basis of sequence similarity. [d]Ratio = log_2Cy5/Cy3. [e]Standard deviation. [f]ND, No data.

cress might account for the relatively fewer salt stress-inducible genes compared to those of Arabidopsis with similar ratios.

Verification of Expression Profiles by RNA Gel-Blot Analysis

We performed RNA gel-blot analysis on expression profiles of *SOS1* and *PDF1.2* genes to confirm the validity of the microarray analyses (Fig. 5). The expression levels of *SOS1* and *PDF1.2* in salt cress were higher than those of Arabidopsis by RNA gel-blot analysis as well as the microarray analysis. Especially, the expression level of *PDF1.2* was very high in salt cress under a normal growth condition or salinity stress, whereas that in Arabidopsis was not detectable under these conditions. Therefore, the expression data obtained by microarray analysis were in good

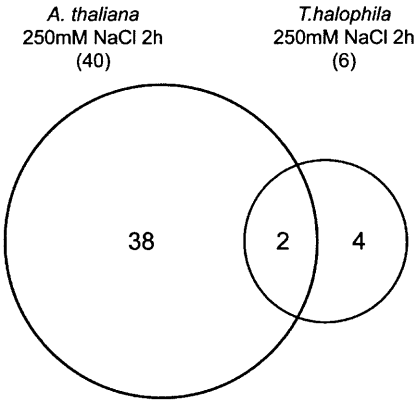

Figure 3. Classification of salt stress-inducible genes in salt cress and Arabidopsis. The salt stress-inducible genes identified were categorized into three groups: (1) genes induced in Arabidopsis; (2) genes induced in salt cress; and (3) genes induced in both Arabidopsis and salt cress. The genes with expression $\log_2$ ratios (salt stressed/unstressed) greater than 1.5 times the average of the three experimental sets were regarded as salt stress-inducible genes.

agreement with those obtained by RNA gel-blot analysis. When we performed RNA gel-blot analysis of other genes, such as *P5CS* using *ThP5CS* cDNA (*AtP5CS* ortholog in salt cress [accession no. BM985832]), the expression of *P5CS* was higher in salt cress. However, when we used Arabidopsis P5CS cDNA as a probe, we could not detect higher expression of *P5CS* in salt cress (data not shown). The disagreement between the microarray analysis and the RNA gel-blot analysis may have arisen because of the difference in their hybridization efficiency and gene family.

ABA and Pro Contents in Salt Cress

AtNCED2 and *AtP5CS* genes were expressed at higher levels in nonstressed salt cress. *NCED* and *P5CS* encode key enzymes of ABA and Pro biosynthesis, respectively. Thus, we measured the accumulation of endogenous levels of ABA and Pro in salt cress and Arabidopsis under normal growth conditions in a hydroponic culture. The ABA content of salt cress was slightly higher than that of Arabidopsis (Fig. 6A). The Pro content in salt cress was markedly higher than that of Arabidopsis during normal growth conditions in a hydroponic culture. Therefore, the extreme stress tolerance to high salinity characteristic of salt cress is due in part to the overaccumulation of Pro under unstressed conditions.

Salt Cress Is Tolerant to Oxidative Stress

The *SOD* gene was expressed at much higher levels in nonstressed salt cress, suggesting that salt cress is tolerant to oxidative stress. SOD is one of the key enzymes in the protective system against oxidative stress since it catalyzes the dismutation of the superoxide radical to O_2 and H_2O_2, a reaction that constitutes the first cellular defense against many oxidative stress situations. To analyze the oxidative tolerance in salt cress, we examined the paraquat tolerance of salt cress and Arabidopsis. Paraquat is known to cause the formation of a superoxide anion and a singlet oxygen (Lin and Culotta, 1995), hence producing oxidative stress in plant cells. Arabidopsis and salt cress plants were germinated and grown vertically on normal Murashige and Skoog (MS) agar medium without paraquat for 7 d and then transferred onto MS agar medium either with or without application of 1 μM paraquat. Without paraquat application, salt cress roots grew more slowly than Arabidopsis roots. However, in the presence of 1 μM paraquat for 7 d, the elongation of Arabidopsis roots stopped and the resultant seedling accumulated a large amount of anthocyanin, whereas the roots of salt cress continued elongation and the seedlings did not accumulate anthocyanin (Fig. 7, A and B). Therefore, compared to Arabidopsis, salt cress was more tolerant not only to high salinity but also to oxidative stress. Oxidative tolerance of salt cress may be in part due to the overexpression of *SOD* genes under normal growth conditions.

DISCUSSION

It is now hypothesized that the mechanisms of salt tolerance in halophytes are substantially the same as those known to exist in glycophytes and that subtle differences in regulation result in large variations in tolerance or sensitivity (Zhu, 2001). In this study, we investigated the strategy used for salt tolerance in a halophyte, salt cress, which is a close relative of Arabidopsis, by using the genomic information of Arabidopsis. Comparison of EST sequences from Arabidopsis and salt cress revealed a high DNA sequence identity (90%–95%) for the majority of transcripts, which indicates that the full-length cDNA microarray of Arabidopsis can be used for expression profiling of salt cress genes. As a result of microarray analyses, only 6 genes were shown to be strongly induced in response to high salinity stress in salt cress, whereas 40 genes were identified as salt stress-inducible genes in Arabidopsis (Tables I and II; Fig. 3). Many abiotic or biotic stress-inducible genes with various functions were expressed under normal growth conditions in salt cress than in Arabidopsis. This may be due to only six inducible genes detected in salt cress. Especially, the tendency observed in the genes up-regulated to high levels, and five of the eight genes with $\log_2$ ratios greater than 2.5 were known to be important genes in abiotic stress or biotic stress tolerance. As a result of hierarchical clustering among the genes highly expressed in salt cress without stress and the stress-inducible genes in Arabidopsis under different stress conditions, it was revealed that one-half of the genes highly expressed in salt cress belong to groups of

Table III. *Genes up-regulated in salt cress grown at normal conditions compared with Arabidopsis*

Clone Name[a]	MIPS[b]	Gene[c]	Log_2 Ratio[d]	SD[e]	Group[f]
RAFL02-01-K01	At4g25100	Superoxide dismutase, SOD[g]	4.10	0.45	V
RAFL09-13-C16	At1g32900	Granule-bound starch synthase-like protein	3.09	0.63	V
RAFL08-17-C21	At1g54010	Myrosinase-associated protein	2.89	0.25	II
ND[h]	At4g19170	9-cis-epoxycarotenoid dioxygenase, AtNCED2[g]	2.87	0.47	V
RAFL04-12-G16	At2g43570	Putative chitinase[g]	2.79	0.13	III
RAFL06-82-G15	At5g44420	Antifungal protein-like, PDF1.2[g]	2.75	0.64	III
RAFL04-12-I10	At3g06660	Hypothetical protein	2.61	0.56	IV
RAFL05-20-O23	At2g39800	Putative Δ-1-pyrroline 5-carboxylase synthetase, AtP5CS1[g]	2.47	0.26	I
RAFL07-13-P10	At5g58540	Ser/Thr-specific protein kinase NPK15, *Nicotiana tabacum*	2.31	0.13	IV
RAFL07-12-I18	At2g45290	Putative transketolase precursor	2.27	0.24	V
RAFL05-18-M07	At4g02280	Putative Suc synthetase	2.24	0.46	I
RAFL09-06-E16	At2g26080	Putative Gly dehydrogenase	2.22	0.16	V
RAFL04-15-P13	At4g17260	Lactate dehydrogenase, LDH1	2.18	0.1	III
RAFL07-18-C20	At2g21330	Fructose bisphosphate aldolase-like protein	2.16	0.09	V
RAFL08-16-N03	At1g09970	Leu-rich repeat receptor-like kinase	2.16	0.19	III
RAFL07-10-O06	At4g33010	P-protein[g]	2.16	0.31	V
RAFL09-09-I16	At5g13630	Cobalamin biosynthesis protein	2.08	0.21	V
RAFL05-05-K17	At3g03780	Met synthase-like protein	2.08	0.15	II
RAFL07-13-A16	At5g67030	Zeaxanthin epoxidase	2.02	0.13	I
RAFL04-13-A11	At2g29340	Tropinone reductase-like protein	2.02	0.19	III
RAFL05-11-A02	At4g14030	Selenium-binding protein-like	2.02	0.03	III
RAFL08-18-C10	At2g21330	Fructose bisphosphate aldolase-like protein	2.00	0.07	V
RAFL04-15-M13	At5g56030	HEAT SHOCK PROTEIN 81-2, HSP81-2	1.96	0.11	III
RAFL07-08-F20	At3g24800	E3 ubiquitin ligase, PRT1	1.96	0.64	IV
RAFL05-17-G21	At5g58330	NADP-dependent malate dehydrogenase	1.95	0.4	V
RAFL06-09-C11	At4g23190	Ser/Thr kinase-like protein	1.95	0.3	III
RAFL09-15-F15	At4g30190	H^+-transporting ATPase type 2, plasma membrane	1.94	0.62	II
RAFL11-07-C13	At4g27470	Putative RING zinc finger protein	1.91	0.25	IV
RAFL05-11-L07	At3g27690	Putative chlorophyll *a*- *b*-binding protein	1.9	0.34	V
RAFL09-12-L07	At4g01950	Predicted protein of unknown function	1.9	0.17	III
RAFL02-01-D07	At3g14990	4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein, putative	1.89	0.15	III
RAFL09-17-L12	At1g62180	Putative adenosine-5′-phosphosulfate reductase	1.88	0.55	III
RAFL09-15-M21	At5g17310	UDP-glucose pyrophosphorylase	1.86	0.16	IV
RAFL09-06-L09	At3g12580	Heat shock protein 70	1.86	0.61	III
RAFL05-18-L03	At1g06430	Cell division protease FtsH, putative	1.86	0.25	I
RAFL04-16-H01	At1g14960	Major latex protein, putative	1.84	0.33	V
RAFL05-21-D08	At3g18490	Putative chloroplast nucleoid DNA-binding protein	1.81	0.4	V
RAFL05-16-C09	At3g54400	Nucleoid DNA-binding-like protein	1.81	0.26	II
RAFL08-13-D07	At1g66280	β-glucosidase, putative[g]	1.8	0.33	II
RAFL09-06-M16	At2g01980	Na^+/H^+ antiporter, SOS1[g]	1.78	0.61	III
RAFL09-07-L16	At2g39210	Nodulin-like protein	1.78	0.5	III
RAFL08-18-C03	At5g63640	Unknown protein	1.77	0.82	III
RAFL04-09-L18	At3g60900	GPI-anchored protein, Fla10	1.77	0.29	V
RAFL05-09-D06	At5g13490	Adenosine nucleotide translocator	1.77	0.37	III
RAFL04-16-G14	At1g44446	Chlorophyll *a* oxygenase	1.77	0.6	V
RAFL07-12-M09	At2g21330	Fructose bisphosphate aldolase-like protein	1.76	0.21	V
RAFL09-13-P07	At3g53130	Cytochrome P450-like protein	1.75	0.33	V
RAFL07-15-N09	At4g04020	Putative fibrillin	1.75	0.17	I
RAFL09-13-N23	At2g21410	Putative vacuolar proton-ATPase subunit	1.71	0.4	III
RAFL06-08-O12	At5g15780	Pro-rich protein	1.69	0.28	V

(*Table continues on following page.*)

Table III. (*Continued from previous page.*)

Clone Name[a]	MIPS[b]	Gene[c]	Log_2 Ratio[d]	SD[e]	Group[f]
RAFL09-10-F11	At5g13630	Cobalamin biosynthesis protein	1.69	0.45	V
RAFL11-03-D07	At5g56000	Heat shock protein	1.67	0.35	III
RAFL11-11-M23	At4g12470	PEARLI 1-like protein	1.66	0.42	II
RAFL09-06-A02	At3g59290	Epsin-like protein	1.66	0.38	III
RAFL09-07-O19	At1g58270	Unknown protein	1.66	0.57	I
RAFL08-13-B17	At1g53280	4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein, putative	1.66	0.2	III
RAFL09-09-M16	At1g53310	Putative phospho*enol*pyrovate carboxylase	1.64	0.47	III
RAFL04-10-D23	At5g23660	MtN3-like protein	1.62	0.05	III
RAFL04-20-P19	At3g49110	Peroxidase	1.61	0.01	III
RAFL09-12-F24	At5g64940	ABC transporter-like	1.61	0.54	V
RAFL09-06-M11	At2g32950	Photomorphogenesis repressor, COP1	1.6	0.57	IV
RAFL09-16-J07	AT5g06600	Ubiquitin carboxyl-terminal hydrolase	1.59	0.46	III
RAFL08-17-N09	At3g52640	Unknown protein	1.58	0.51	IV
RAFL04-17-N08	At1g63900	Putative RING zinc finger protein	1.58	0.08	IV
RAFL09-10-N21	At3g56200	Amino acid transporter family	1.58	0.42	III
RAFL05-21-G06	At2g18960	Plasma membrane proton ATPase, PMA	1.58	0.17	III
RAFL08-16-K20	AT4g21990	5′-adenylylsulfate reductase, APR3	1.57	0.6	III
RAFL07-08-K14	At3g09440	Heat-shock protein, At-hsc70-3	1.56	0.05	III
RAFL05-16-I09	At5g20830	Sucrose-UDP glucosyltransferase	1.56	0.58	I
RAFL09-13-F15	At1g71270	ARE1-like protein	1.55	0.47	IV
RAFL05-02-M09	At3g61820	Unknown protein	1.54	0.12	V
RAFL06-09-I16	At5g16570	Gln synthetase	1.54	0.52	II
RAFL11-13-K15	At1g60470	Galactinol synthase, AtGolS4	1.54	0.76	I
RAFL04-20-A09	At3g16370	Putative APG protein	1.54	0.11	V
RAFL09-09-D18	At3g17650	Unknown protein	1.53	0.15	II
RAFL05-07-K16	At4g28250	Putative β-expansin/allergen protein	1.53	0.38	V
RAFL07-15-O10	At2g39210	Nodulin-like protein	1.53	0.42	III
RAFL09-14-N06	At2g04030	Heat shock-like protein	1.52	0.18	V
RAFL07-10-M10	At4g11420	Initiation factor 3a-like	1.52	0.37	III
RAFL08-18-M13	At2g38110	Phospholipid/glycerol acyltransferase family	1.51	0.75	III
RAFL04-19-A22	At5g08650	GTP-binding protein LepA homolog	1.5	0.33	V
RAFL05-11-D11	At1g55490	Rubisco subunit binding-protein β subunit	1.5	0.11	V
RAFL08-10-G22	At4g02280	Putative sucrose synthetase	1.5	0.76	I

[a]RIKEN Arabidopsis full-length (RAFL) cDNA. [b]MIPS protein code. [c]Indicates the putative functions of the gene products that are expected on the basis of sequence similarity. [d]Ratio = log_2Cy5/Cy3. [e]Standard deviation. [f]Five groups: I: abiotic stress inducible genes, II: biotic stress inducible genes, III: both abiotic and biotic stress inducible genes, IV: abiotic and biotic stress non-inducible genes, V: abiotic and biotic stress supressible genes (see Fig. 4). [g]genes discussed in manuscript. [h]ND, No data.

genes that were abiotic, biotic, or abiotic and biotic stress-inducible in Arabidopsis (Fig. 4A). Furthermore, the expression profiles of genes highly expressed in salt cress under normal growth conditions resembled those of Arabidopsis genes induced under abiotic stresses rather than biotic stresses (Fig. 4B). However, some important genes involved in biotic stress tolerance were also observed in genes highly expressed in salt cress without stress. These observations suggest that salt cress is tolerant to biotic stresses as well as abiotic stresses because of constitutive overexpression of stress-related genes under unstressed conditions. These results indicate that salt cress does not immediately respond to a variety of environmental stresses, including high salinity at the transcriptional level, but instead adapted to them by expressing a number of genes that are known components of abiotic or biotic stress defense and survival machinery even in the absence of these stresses (Table III; Fig. 4). The genes overexpressed in the salt cress under normal conditions include *Fe-SOD*, *P5CS*, *PDF1.2*, *AtNCED*, *P-protein*, β-glucosidase, and *SOS1* (see Table III). Possible functions of their gene products in stress tolerance of salt cress are discussed in the following.

Pro, the most common osmoprotectant, accumulates in many organisms, including higher plants exposed to environmental stresses such as high salinity, drought, and freezing. Some reports have even indicated a positive correlation between Pro accumulation and the acquisition of stress tolerance (Kavi Kishor et al., 1995; Nanjo et al., 1999a, 1999b). Since the Pro synthesis gene *AtP5CS* was up-regulated in salt cress under normal conditions, the Pro content of

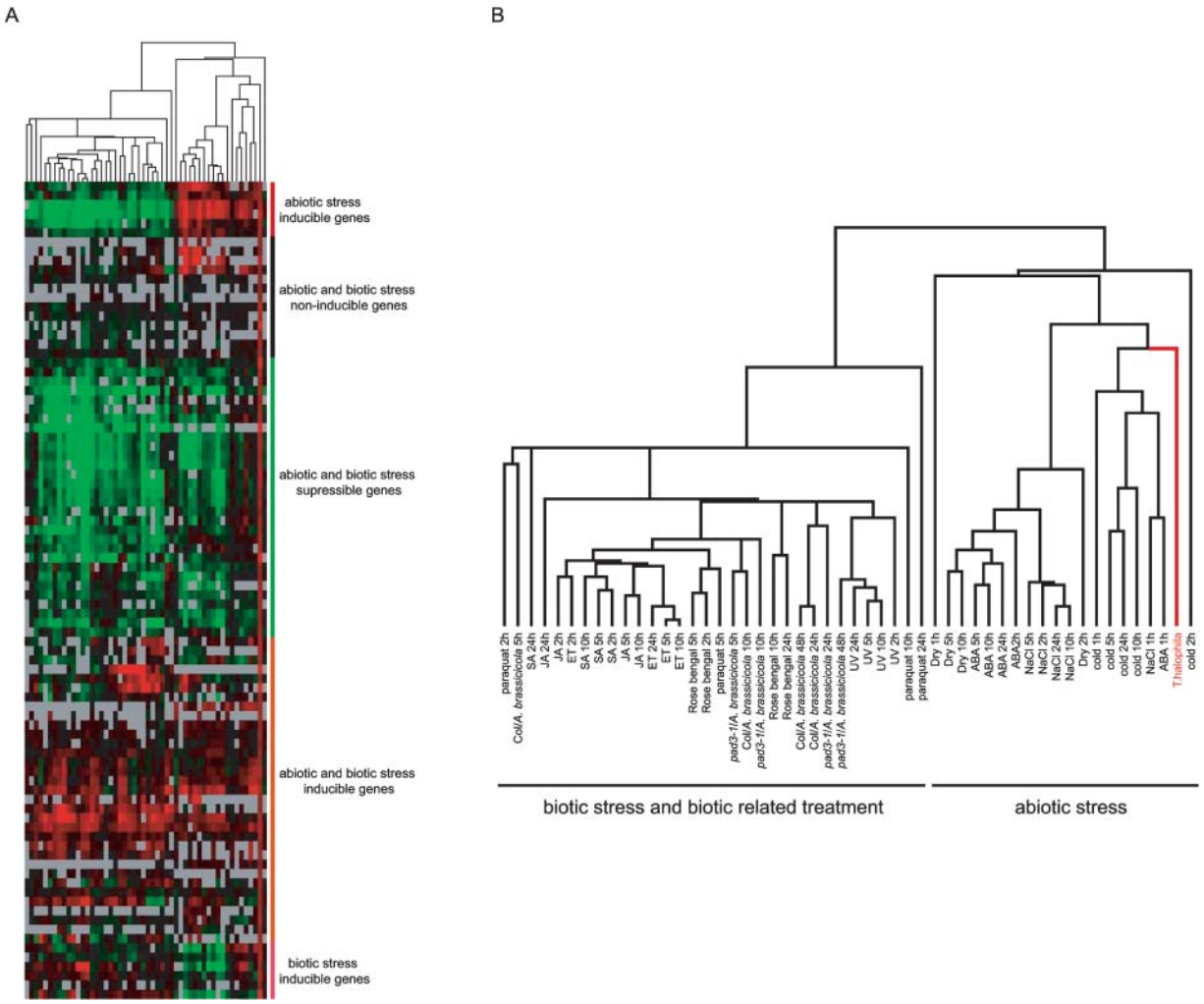

Figure 4. Hierarchical clustering among the genes highly expressed in salt cress and the results of microarray analyses that included various abiotic stress treatments or various biotic stress and biotic-related treatments of Arabidopsis using this Arabidopsis cDNA microarray. A, Overview of the hierarchical cluster display. The fold change values for each sample, relative to untreated control samples, were $\log_2$ transformed and subjected to complete linkage hierarchical clustering. Expression values higher and lower than those of the control are shown in red and green, respectively. As absolute value of fold difference increased, the color intensity increased. The bars at the right end indicate five rough groups: abiotic, biotic, both abiotic and biotic stress-inducible genes, abiotic and biotic stress-suppressible genes, and abiotic and biotic stress-noninducible genes. B, The expansion figure of A. The relationship among experiments across all of the genes included in the cluster analysis and the type of each experiment are indicated (for details, see Seki et al., 2002a, 2002b; Narusaka et al., 2003).

salt cress and Arabidopsis plants was measured under normal conditions to confirm the accuracy of the microarray analysis. Salt cress was found to accumulate significantly more Pro than Arabidopsis. The amount of Pro in salt cress was much higher than that in Arabidopsis under high salt stress, and equal to that in antisense transgenic Arabidopsis plants known to exhibit improved salt tolerance with an *AtProDH* cDNA encoding Pro dehydrogenase, which catalyzes Pro degradation (Nanjo et al., 1999a). These results suggest that the result of microarray analysis that *AtP5CS* was up-regulated is in good agreement with the phenomenon of excess accumulation of Pro. An external supply or extreme accumulation of Pro has also been reported to be toxic to plants in spite of its protective functions under stress (Hellmann et al., 2000; Deuschle et al., 2001; Mani et al., 2002; Nanjo et al., 2003). Growth of the salt cress seedling is slower than that of Arabidopsis. This phenomenon may be induced, in part, by the overaccumulation of Pro under normal growth conditions because a high level of Pro has a negative effect on plant growth (Nanjo et al., 2003).

The *Fe-SOD* gene was overexpressed in salt cress under normal conditions, and salt cress showed a higher tolerance to paraquat treatment (Fig. 6). SODs are essential components in almost all plant antioxidant defense mechanisms. The SOD isoenzymes can be

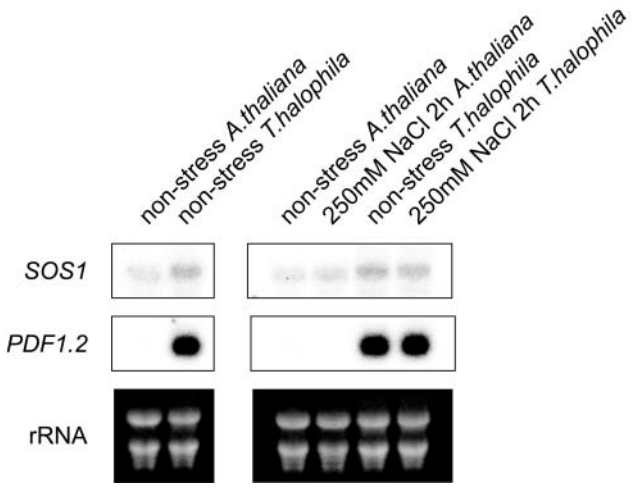

Figure 5. RNA gel-blot analysis of *AtP5CS, ThP5CS, SOS1,* and *PDF1.2* genes in salt cress and Arabidopsis. Total RNA was prepared from 3-week-old Arabidopsis and 4-week-old salt cress plants that were the same in size during nonstress or 250 mM NaCl stress using the microarray analyses. Each lane was loaded with 10 μg of total RNA. The RNA was fractionated on a 1% agarose gel, blotted onto a nylon membrane, and hybridized with ^{32}P-labeled *AtP5CS, ThP5CS, SOS1,* and *PDF1.2* DNA fragments as probes.

classified into Cu/Zn, Mn, and Fe types according to their metal cofactor. Plants generally contain Fe-SOD and/or Cu/Zn-SOD in the chloroplasts (Holmberg and Bulow, 1998). In Arabidopsis ecotype Cvi, a new chloroplastic Cu/Zn-SOD isoenzyme, which was encoded by a Cvi-specific allele, has been identified. Paraquat treatments of Arabidopsis ecotype Landsberg *erecta* and Cvi resulted in higher levels of chloroplastic Cu/Zn-SOD activity in Arabidopsis Cvi, and the relative transcript levels were higher in Cvi under normal growth conditions. In addition, Arabidopsis Cvi showed a higher tolerance to paraquat treatments (Abarca et al., 2001). The mechanisms of oxidative stress tolerance in salt cress are similar to those of Arabidopsis Cvi. Expression of the Arabidopsis *Fe-SOD* gene protected both the plasmalemma and PSII against the superoxide generated by scavenging radicals during leaf discs impregnated with metal viologen (Van Camp et al., 1996). Besides the oxidative stress tolerance, it has been reported that transgenic alfalfa overexpressing *Mn-SOD* exhibited higher tolerance to freezing and water-deficit stresses. Thus, the extreme tolerance to salt stress of salt cress may be supported by the high level expression of the *Fe-SOD* gene in cooperation with *P5CS*.

With the acquisition of salt tolerance, there is no subsequent Na^+ accumulation in the cytoplasm. Theoretically, there are three mechanisms: (1) reduction of Na^+ influx; (2) vascuolar compartmentalization of Na^+; and (3) excretion of Na^+ via plasma membrane Na^+/H^+ antiporters. Overexpression of the vacuolar Na^+/H^+ antiporter *NHX1* has been shown to confer salt tolerance in Arabidopsis and tomato, suggesting the utility of this vascuolar compartmentation of Na^+ (Apse et al., 1999; Zhang and Blumwald, 2001). In addition, overexpression of a plasma membrane Na^+/H^+ antiporter gene in a freshwater cyanobacterium showed extreme salt tolerance and allowed growth in seawater, indicating the effectiveness of excretion of Na^+ via plasma membrane Na^+/H^+ antiporters for salt tolerance (Waditee et al., 2002). We found that less NaCl is accumulated in salt cress plants than in Arabidopsis after 10 or 24 h of treatment (Fig. 2B), and the expression of the *SOS1* gene in salt cress under normal conditions is higher than that of Arabidopsis (Table III). Recently, overexpression of *SOS1* was shown to confer salt tolerance by retrieving Na^+ from the xylem of transgenic plants (Shi et al., 2003). These results suggest that the higher expression of *SOS1* may support excretion of Na^+ from the xylem in salt cress, thereby limiting Na^+ accumulation in the shoot in salt

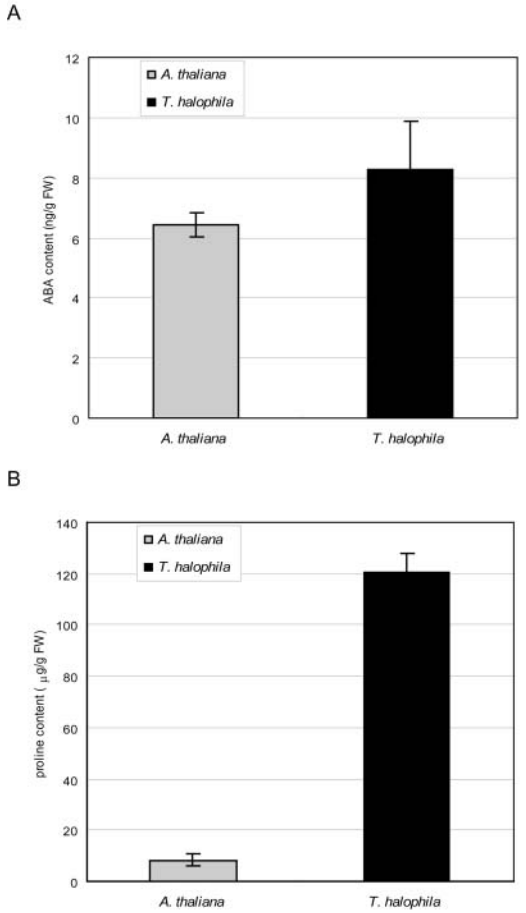

Figure 6. ABA and Pro contents in salt cress and Arabidopsis. A, Accumulation of ABA in the entire plant of salt cress and Arabidopsis during normal growth conditions in hydroponic culture. Three-week-old Arabidopsis and 4-week-old salt cress plants grown under normal conditions were used for the analysis. B, Pro content in the entire plant of salt cress and Arabidopsis during normal growth conditions in hydroponic culture. Three-week-old Arabidopsis and 4-week-old salt cress plants grown under normal conditions were used for the analysis.

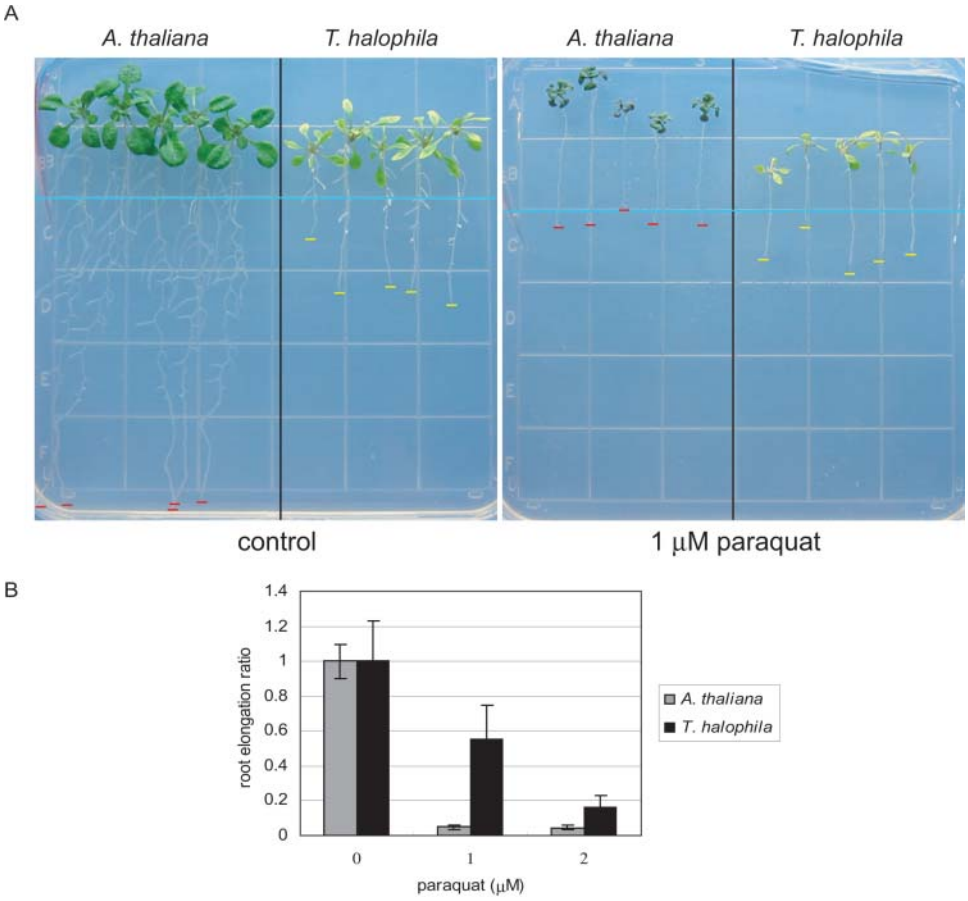

Figure 7. Oxidative stress tolerance of salt cress. A, Root elongation assay with paraquat. Salt cress and Arabidopsis seedlings were grown on vertical MS plates containing 1.2% (w/v) agar and 3% Suc. One week after germination, the seedlings were transferred onto either the control MS agar plates (left) or MS agar plates containing 1 μM paraquat (right). The root tips of the seedlings were arranged uniformly along the light-blue line to ensure accurate measurements. Red and yellow bars show root ends of salt cress and Arabidopsis. The pictures were taken 10 d after the seedlings were transferred. B, Root elongation ratio in salt cress (black bar) and Arabidopsis (gray bar) during the exogenous paraquat treatment. The SD of 10 replicated measurements is indicated.

cress during high salt stress. The *SOS1* transcript in the overexpressed transgenic lines is present at only a slightly higher level than in the wild type under normal growth conditions, whereas compared with the wild type the level is much higher under salt stress. This suggests that *SOS1* mRNA is unstable in the absence of salt stress in most or all cells and that salt stress causes a posttranscriptional stabilization of the transcript (Shi et al., 2003). However, in salt cress, the *SOS1* gene is expressed at high levels even in the absence of stress conditions. In salt cress, a number of abiotic or biotic stress-inducible genes were expressed under normal growth conditions (Table III). Therefore, the machinery for degrading the *SOS1* transcripts in salt cress may be down-regulated in normal growth conditions.

Interestingly, in salt cress, not only abiotic but also biotic stress-inducible genes were overexpressed under normal growth conditions (Table III). Pathogenesis-related protein *PDF1.2*, used as a general marker gene for pathogenesis, chitinase, P-protein, and β-glucosidase were expressed at higher levels in salt cress. Furthermore, the comparison of the genes expressed at higher levels in salt cress with our previous microarray analyses of various biotic or biotic-related treatments (Narusaka et al., 2003) revealed a number of genes induced by biotic stress or oxidative stress treatments (Fig. 4). Plant defense responses to microbial attack are regulated through a complex network of signaling pathways that involve three signaling molecules: SA, JA, and ET. The SA and JA signaling pathways are mutually antagonistic (Kunkel and Brooks, 2002). *PDF1.2*, chitinase, and β-glucosidase function in the JA and ET signaling pathways, and their expressions are induced by JA and ET but not SA (Penninckx et al., 1998; Hara-Nishimura and Matsushima, 2003). Incidentally, the P-protein gene was also expressed at higher levels in salt cress. Very recently, Chandok et al. (2003) demonstrated that the pathogen-induced NO-synthesizing enzyme is a variant form of P-protein of Gly decarboxylase. After an attack by a pathogen, NO induces the expression of PR-1 through a SA signaling pathway (Wendehenne et al., 2001). However, the P-protein is not a SA-inducible gene, and PR genes are not expressed at a high level in salt cress. In addition, in the cluster analysis shown in Figure 4, the branch of salt cress is close to the branch of JA and ET rather than to that of SA. This suggests that JA and ET signaling might be up-regulated in salt cress under normal growth condition.

In conclusion, we showed that salt cress had not only salt stress tolerance but also oxidative stress

tolerance. Based on expression profiling using the full-length Arabidopsis cDNA microarray, we showed that various genes induced by abiotic and biotic stresses in Arabidopsis are overexpressed in unstressed conditions in salt cress. This suggests that stress-inducible signaling pathways are constitutive and active in salt cress even under normal growth conditions without stress.

MATERIALS AND METHODS

Plant Material and Salt Stress Treatments for Soil-Grown Plants

Salt cress (*Thellungiella halophila*) and Arabidopsis L. Heynh., ecotype Columbia, were sown on MS plates containing 1.2% (w/v) agar and 3% Suc. The seeds were stratified at 4°C for 7 d and then transferred to 22°C under continuous light for germination and growth. Two or 3 weeks after germination, seedlings of Arabidopsis and salt cress were transferred onto separate 9-cm plastic pots filled with a 1:1 perlite:vermiculite and watered with 1,000-fold diluted Hyponex (Hyponex, Osaka). One week after transfer, the seedlings were transferred into a 1,000-fold diluted Hyponex solution containing 500 mM NaCl for the salinity stress treatment.

Salinity Treatments in Hydroponic System

In addition to the above method, two- or 3-week-old seedlings of Arabidopsis and salt cress were transferred onto tea strainers filled with glass beads with a hydroponic culture (1,000-fold diluted Hyponex). One week after transfer, the tea strainers containing the seedlings were transferred into 1,000-fold diluted Hyponex containing 250 mM NaCl for salinity stress.

Measurement of Na^+ Content

Three-week-old Arabidopsis and 4-week-old salt cress plants grown in the hydroponic system were exposed to 250 mM NaCl solution for 2, 5, 10, and 24 h. We sampled only rosette leaves because this portion was immersed in the hydroponic culture. Na^+ content was determined by converting the result of electrolyte leakage from leaves into the Na^+. A total of 0.1 g leaves from each treatment group were immersed in 5 mL of sterile distilled water. The solution was measured for conductivity with a conductivity meter (DS-15; Horiba, Kyoto) after the sample was boiled for 14 min.

Measurement of Pro Content

Three-week-old Arabidopsis and 4-week-old salt cress plants grown in the hydroponic system were ground in distilled water. The homogenate was boiled for 6 min and then centrifuged at 10,000*g* for 15 min at 4°C. The supernatant was precipitated with 10% TCA for 5 h and centrifuged at 10,000*g* for 20 min. The supernatant after TCA precipitation was derivatized, and the contents of free Pro were determined by HPLC (model LC Module I plus; Waters, Milford, MA).

Measurement of ABA Content

Three-week-old Arabidopsis and 4-week-old salt cress plants grown in the hydroponic system were used as samples. Endogenous ABA was measured using exactly the same procedure as previously described (Iuchi et al., 2000).

RNA Isolation and RNA Gel-Blot Analysis

Total RNA was prepared using TRIZOL reagent (Life Technologies, Rockville, MD), and mRNA was prepared using an mRNA isolation kit (Miltenyi Biotec, Auburn, CA). Isolated total RNA was also used for RNA gel-blot analysis. Total RNA was fractionated in a 1% agarose gel containing formaldehyde and was blotted onto a nylon filter. The filters were hybridized with ^{32}P-labeled fragments at 57°C, washed twice with 0.1 × SSC, 0.1% SDS, at 60°C for 15 min, and autoradiographed.

Full-Length cDNA Microarray Analysis

Full-length cDNA microarray analysis was carried as reported previously (Seki et al., 2001, 2002b). In this study, we used a cDNA microarray containing approximately 7,000 Arabidopsis genes (Seki et al., 2002b). mRNAs from salt cress plants and Arabidopsis plants were used for preparation of Cy3-labeled and Cy5-labeled cDNA probes, respectively. These cDNA probes were mixed in equal amounts and hybridized with the cDNA microarray.

Data Analysis

Image analysis and signal quantification were performed with QuantArray version 2.0 (GSI Lumonics, Oxnard, CA). Background fluorescence was calculated on the basis of the fluorescence signal of the negative control genes, the mouse nicotinic acetylcholine receptor epsilon-subunit (nAChRE) gene and the mouse glucocorticoid receptor homolog gene. Intensity-dependent normalization (Yang et al., 2002) was used to equalize hybridization signals generated from different samples. Gene-clustering analysis was performed with Cluster/TreeView (Eisen et al., 1998). Average linkage clustering with uncentered correlation was used.

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