Original Paper Plant Biotechnology, **21** (2), 127-133 (2004)

Isolation and Characterization of Sea Aster Salt-Stress Responsive Cysteine Protease Gene Obtained by a Hetero-probed Macroarray

Migiwa TAKEDA[1], Chikara KUWATA[3a], Yuichi UNO[2]*, Michio KANECHI[1], Daisuke SHIBATA[3] and Noboru INAGAKI[1]

[1]*Graduate School of Science and Technology, Kobe University, Hyogo 657-8501, Japan*
[2]*Faculty of Agriculture, Kobe University, Hyogo 657-8501, Japan*
[3]*Kazusa DNA Research Institute, Chiba 292-0818, Japan*
[a]*Present Address: Chiba Prefectural Agriculture Research Center, Chiba 266-0006, Japan*
**Corresponding author* E-mail address: yuno@kobe-u.ac.jp

Received 28 December 2003; accepted 15 January 2004

Abstract

To isolate and analyze salt-stress inducible genes in a halophyte, sea aster (*Aster tripolium* L.), we screened 5760 Arabidopsis cDNA clones by macroarray procedure using ^{33}P-labeled cDNA targets synthesized from mRNAs isolated from NaCl treated and untreated sea aster seedlings. Seventeen Arabidopsis cDNAs were hetero-hybridized to NaCl inducible sea aster genes. These cDNAs were used as probes to isolate cDNA homologs from a sea aster cDNA library. One of the obtained cDNAs shared 71% amino acids identity with Arabidopsis cysteine protease (AtCysP) and named SaCysP (sea aster CysP). Northern blot analysis revealed that mRNAs corresponding to both SaCysP and AtCysP were induced by salt and osmotic stress in leaves. On the other hand, SASR21 mRNA encoding another CysP in sea aster was irresponsive to these stress in leaves but respond in roots. SaCysP and SASR21 genes may have a tissue-specific function in stress response by modulating their expression levels.

Key words: Arabidopsis, Cysteine protease, Gene expression, Macroarray, Mannitol, NaCl stress, Sea aster (*Aster tripolium* L.).

Abbreviations

CysP, cysteine protease; SASR, sea aster stress responsive.

Introduction

Salt stress is one of the most severe limiting factors of plant growth and crop production, by both toxicity of some ions and dehydration, similar to osmotic stress. The halophytes, which grow naturally in elevated levels of Na^+, have strong tolerance to salinity and compensations for such conditions at morphological and physiological levels (Orcutt and Nilsen, 1996). Sea aster (*Aster tripolium* L.) is an annual halophyte that grows naturally in salt marsh and coastal areas. The growth of this plant is not inhibited by 150 mM NaCl stress but is affected by 300 mM NaCl stress in both whole plants (Uno *et al.*, 1996a) and suspension-cultured cells (Uno *et al.*, 1996b). Sea aster may utilize the Na^+ for osmoregulation to decrease leaf osmotic potential under NaCl stress (Matsumura *et al.*, 1998).

In a previous report, we isolated and characterized nine SASR (sea aster stress responsive) clones using a differential screening method from sea aster seedlings treated with 400 mM NaCl (Takeda *et al.*, 2003). All mRNAs corresponding to these SASR genes were accumulated under high salt conditions and the transcripts of 3 out of 9 genes were induced by osmotic stress. Differential screening technique has been used successfully to clone genes that are regulated under environmental stress conditions (Yamaguchi-Shinozaki *et al.*, 1992; Gosti *et al.*, 1995; Iuchi *et al.*, 1996). However, it is known that this method is unsuitable for detection of differentially expressed genes with low transcript abundance. Furthermore, the information resulted from a differential screening depends considerably on the quality of the cDNA library in a condition that the plant material has not been fully provided with the entire genome sequences or expressed sequence tag (EST) clones. In the model plant Arabidopsis, the entire genomic sequences have been determined (The Arabidopsis genome Initiative, 2000) and EST projects have also provided a major contribution

with the discovery of expressed genes (Newman *et al.*, 1994; Cooke *et al.*, 1996; Asamizu *et al.*, 2000). The recent developments in cDNA array technology offer more efficient alternative to the library screening for detection of differentially expressed clones, as the expression profiles of multiple cDNA fragments are generated simultaneously through a single hybridization. In fact, expression profiles of many genes were analyzed under various water-related stress conditions in Arabidopsis (Seki *et al.*, 2001, 2002a, 2002b; Bray, 2002; Kreps *et al.*, 2002; Oono *et al.*, 2003). However, it has not been well analyzed with cDNA array about unique plants such as halophytes because of their undeveloped genome-informative study.

In this study, we tried to utilize Arabidopsis cDNA macroarray for screening and expression analysis of salt-inducible genes in sea aster by using a hetero-probing method. One of the obtained cDNA clones encodes a protein with sequence similarity to cysteine protease (CysP). CysPs are widely distributed in plants, animals and microorganisms. The genes encoding CysPs have been shown to respond to environmental stress, such as cold or desiccation (Schaffer and Fischer, 1988; Guerrero *et al.*, 1990; Koizumi *et al.*, 1993). It is important to compare the expression profiles of homologous genes in a halophyte and a glycophyte for understanding the different adaptation mechanisms between them. Here, we analyzed the expression of CysP genes from sea aster and Arabidopsis under salt and osmotic stress conditions.

Materials and Methods

Plant materials and stress treatments

Seeds of sea aster were germinated in a vermiculite bed and irrigated with 0.1% Hyponex (N:P:K=1:2:1, Hyponex, Osaka, Japan) under approximately 200 μmol m^{-2} s^{-1} illumination and 23℃ with a day/night cycle of 16/8 h in a chamber. Seedlings with three to four foliage were transferred to a hydroponic condition with 0.1% Hyponex. For macroarray analysis, sea aster plants were treated with 400 or 0 mM NaCl for 10 h after 10 days of hydroculture. Total RNA was purified from whole plants as described by Sambrook *et al.* (1989).

For stress-tolerant test, three-week-old sea aster and *Arabidopsis thaliana* (Columbia ecotype) plants were grown hydroponically with 0.1% Hyponex containing 0 mM, 200 mM NaCl or 400 mM mannitol (equal to osmotic pressure for 200 mM NaCl) at 23℃ for 4 days under dimlight.

For Northern blot analysis of salt or osmotic stress treatments, eight-week-old sea aster plants and three-week-old Arabidopsis plants were subjected to the same stress treatments for 0, 1, 3, 6, 12, and 24 h. Leaves and roots were harvested, frozen in liquid nitrogen and stored at -80℃. Control plants were subjected to 0.1% Hyponex and harvested at the same time as the stressed plants.

Hybridization of cDNA macroarray filters

The cDNA clones used for macroarray filters had been prepared from above-ground organs of Arabidopsis plants (Asamizu *et al.*, 2000). Inserts of EST clones were amplified by PCR and spotted onto two nylon filters (8x12 cm each) as described by Sasaki *et al.* (2001). Each EST and λDNA (negative control) were spotted in duplicate on the filter.

Poly(A)$^+$ RNAs of sea aster were purified from the total RNA of salt-treated or untreated whole plants using oligo(dT) cellulose columns (Life Technologies, U.S.A.). Each mRNA sample was

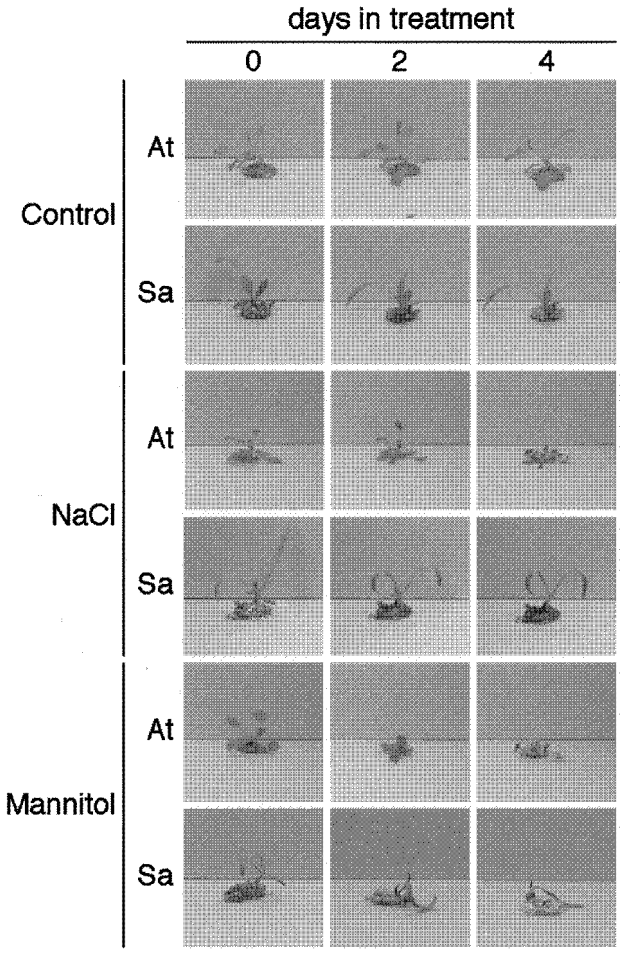

Fig. 1 Comparison of tolerance to salt or osmotic stress between sea aster and Arabidopsis. Three-week-old sea aster and Arabidopsis plants were supplemented with 0 mM (Control), 200mM NaCl (NaCl), or 400 mM mannitol (Mannitol), and then allowed to grow hydroponically for 4 days. At, Arabidopsis plants; Sa, sea aster plants.

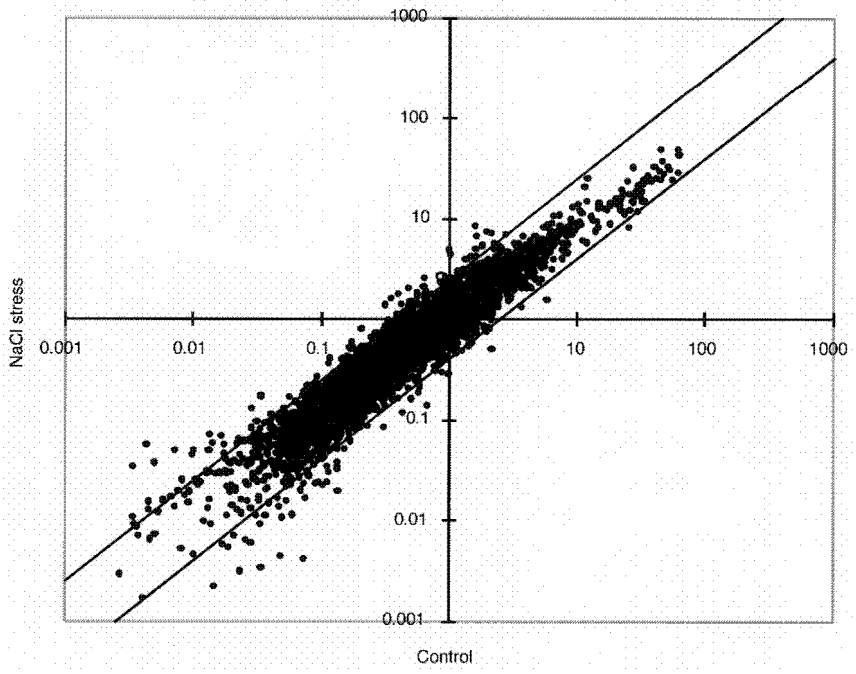

Fig. 2 A scattered plot of expression ratio obtained from macroarray analysis. The expression ratio of each spot in control sample (Control, abscissa axis) was plotted against that in 400 mM NaCl-treated sample (NaCl stress, ordinate axis). The cDNA macroarray containing Arabidopsis ESTs and negative control clones (λ DNA) hybridized with ^{33}P-labeled target cDNAs of sea aster. The upper and lower black lines show 2.5 fold elevation and decline changed by the NaCl-treatment, respectively. The open circle shows a spot corresponding to a cysteine protease gene.

reverse-transcribed in a reaction mixture containing 0.1 or 1 μg of mRNA using ThermoScript RT-PCR kit (Gibco BRL) in the presence of [α-^{33}P]dCTP, incubated at 50℃ for 60 min.

Hybridization with the labeled targets was carried out in the presence of 0.5 M Na_2HPO_4, 1 mM EDTA, and 7% SDS at 50℃. After incubation, the filters were washed in 1x SSC, 0.1% SDS at 55℃ for 15 min and twice in 0.5x SSC, 0.1% SDS at 55℃ for 15 min each, then exposed to an imaging plate (Fuji Film, Japan) for detection. Hybridization was conducted twice.

Identification of salt stress-responsive genes

Radioactive images were detected with a scanner (STORM830, Molecular Dynamics, U.S.A.), and quantification of the signal intensity was carried out using an Array Vision (Imaging Research, Canada). The signal intensities of the duplicated spots were averaged, and local backgrounds were calculated for each filter using the intensity of all the λ DNA spots. The backgrounds were subtracted to obtain raw signal intensity. Global normalization was adopted for normalizing the difference of signal intensity of each nylon filter: the raw signal intensity of all spots except for λ DNA spots on the filter was averaged and the relative signal intensity was calculated as the ratio of each signal to the average intensity of each filter. The value thus estimated is called the 'expression ratio.' Data analyses were carried out using Microsoft Excel.

Isolation and sequence analysis of sea aster cDNA clones

Sea aster cDNA library was synthesized with RNA isolated from salt-stressed whole plants (Takeda *et al.*, 2003) and screened by plaque hybridization (approximately $3.0x10^5$ pfu) using the radio-labeled Arabidopsis cDNA probe synthesized with a random primer labeling kit (Roche, Germany). Hybridization was carried out in 1% SDS, 2x SSC, 10% Dextran sulphate and 50% formamide at 42℃. Each membrane was washed twice in 2x SSC, 1% SDS at 55℃ for 20 min each and twice in 0.2x SSC, 1% SDS at 55℃ for 20 min each (low stringency). The obtained positive plaques were subjected to *in vivo* excision to yield pBluescript phagemid with helper phage strain ExAssist (Stratagene, U.S.A.). The cDNA clones were sequenced using an ABI PRISM 310 Genetic Analyzer (ABI, U.S.A.). The obtained sequences were compared with Arabidopsis proteome database using BLAST search programs on the network (http://mips.gsf.de/).

Northern blot analysis

Total RNA was purified from sea aster and

Arabidopsis using sepasol RNA I kit (nakalai tesque, Japan) according to manufacturer's protocol. Twenty μ g of total RNA per lane were subjected to electrophoresis in 1% agarose/formaldehyde gel and transblotted onto a nylon membrane (PALL, U.S.A.) by capillary transfer. Inserted cDNAs encoding putative cysteine protease of sea aster or Arabidopsis were used as radio-labeled probes. Hybridization was carried out in 50% (v/v) formamide, according to the standard method (Sambrook *et al.*, 1989).

Results and Discussion

As a first step to compare the tolerances to salt or osmotic stress in sea aster with those in Arabidopsis, both three-week-old plants were tested for their survival under salt and osmotic stresses by adding 200 mM NaCl and 400 mM mannitol into hydroponic solutions, respectively. Sea aster clearly showed stronger tolerance to salt than Arabidopsis after 4 days treatment (**Fig. 1**). In contrast, both plants showed sensitivity to osmotic stress, wilted and died within 4 days. The tolerance of sea aster to NaCl but not to mannitol suggests that this plant may modulate the high osmotic potential only by accumulating sodium ions to prevent water loss. The high accumulation rate of sodium ions in sea aster treated with NaCl was indicated by several reports (Shennan *et al.*, 1987; Matsumura *et al.*, 1998; Ueda *et al.*, 2003). The different mechanisms of tolerance between sea aster and Arabidopsis

```
                1                                                        60
SaCysP          MN----LLSISSLLLL-LL-ISAVTADSSDPLIRQVVQNDETEIESDPLLDPEHHFKLFK
At4g39090       MD----RLKLYFSVFVLSFFIVSVSSSDVNDGDDLVIR-QVVGGAEPQVLTSEDHFSLFK
At2g21430       MD---YHLRVLFSV-SLIFVFVSVSVCG--D-EDVLIR-QVVDETEPKVLSSEDHFTLFK
At4g16190       MDRVVFFFLIAATLLAGSLGSTVISGEVTDGFVNPIRQVVPEE-NDEQLLNAEHHFTLFK
                *.      .                ..                      *  * ** ***
SaCysP          NKFGRTYDTEEEHEYRLTVFKSNLRRAKRHQVLDPTAKHGVTKFSDLTPSEFRKKYLGLK
At4g39090       RKFGKVYASNEEHDYRFSVFKANLRRARRHQKLDPSATHGVTQFSDLTRSEFRKKHLGV-
At2g21430       KKFGKVYGSIEEHYYRFSVFKANLLRAMRHQKMDPSARHGVTQFSDLTRSEFRRKHLGV-
At4g16190       SKYEKTYATQVEHDHRFRVFKANLRRARRNQLLDPSAVHGVTQFSDLTPKEFRRKFLGLK
                 *... *   .** .*. ***.**.** *.* .**.* ****.***** .*** * **
SaCysP          SK-LKLPADANKAPILPTSNLPQDFDWRDKGAVTPVKNQGSCGSCWSFSTTGALEGSHFL
At4g39090       RSGFKLPKDANKAPILPTENLPEDFDWRDHGAVTPVKNQGSCGSCWSFSATGALEGANFL
At2g21430       KGGFKLPKDANQAPILPTQNLPEEFDWRDRGAVTPVKNQGSCGSCWSFSTTGALEGAHFL
At4g16190       RRGFRLPTDTQTAPILPTSDLPTEFDWREQGAVTPVKNQGMCGSCWSFSAIGALEGAHFL
                  ...** *.. ****** .**  ****. **********.******** .******..**
SaCysP          QTGELVSLSEQQLVDCDHECDPAEYNSCDSGCNGGLMNNAFEYILKAGGLQKEADYPYTG
At4g39090       ATGKLVSLSEQQLVDCDHECDPEEADSCDSGCNGGLMNSAFEYTLKTGGLMKEEDYPYTG
At2g21430       ATGKLVSLSEQQLVDCDHECDPEEEGSCDSGCNGGLMNSAFEYTLKTGGLMREKDYPYTG
At4g16190       ATKELVSLSEQQLVDCDHECDPAQANSCDSGCSGGLMNNAFEYALKAGGLMKEEDYPYTG
                .*. ****************  .  ******.***** **** ** *** ..* ******
SaCysP          RD-GTCKFDKSKIAASVANFSVVSTDEDQIAANLVTNGPLAIGINAAWMQTYIGQVSCPY
At4g39090       KDGKTCKLDKSKIVASVSNFSVISIDEEQIAANLVKNGPLAVAINAGYMQTYIGGVSCPY
At2g21430       TDGGSCKLDRSKIVASVSNFSVVSINEDQIAANLIKNGPLAVAINAAYMQTYIGGVSCPY
At4g16190       RDHTACKFDKSKIVASVSNFSVVSSDEDQIAANLVQHGPLAIAINAMWMQTYIGGVSCPY
                 *   ** *.***.***.****.* .*.******. .**** .***  ******.*****
SaCysP          ICSKTKMDHGVLLVGYGSAGYAPLRFKEKPYWIIKNSWGEDWGEDGYYKLCSG-YNACGM
At4g39090       ICTR-RLNHGVLLVGYGAAGYAPARFKEKPYWIIKNSWGETWGENGFYKICKG-RNICGV
At2g21430       ICSR-RLNHGVLLVGYGSAGFSQARLKEKPYWIIKNSWGESWGENGFYKICKG-RNICGV
At4g16190       VCSKSQ-DHGVLLVGFGSSGYAPIRLKEKPYWIIKNSWGAMWGEHGYYKICRGPHNMCGM
                .*.     *******.*..*... * *************. *** * **.* *  * **
SaCysP          DTMVSAV-VSTNT---   363
At4g39090       DSMVSTVAATVSTTAH   368
At2g21430       DSLVSTVAATTS----   361
At4g16190       DTMVSTV-AAVHTSPK   373
                * .**.* .   .
```

Fig. 3 Comparison of the deduced amino acid sequences of cysteine protease from sea aster with those from Arabidopsis; At4g39090 (RD19), At2g21430, At4g16190 (AtCysP). SaCysP (AB161375) from sea aster was obtained by low-stringency screening using AtCysP (At4g1690) as a probe. Asterisks represent common amino acid recidues among all proteins. Dots indicate that three amino acids are common among all proteins. Boxed sequences indicate a conserved motif of prokaryotic membrane lipoprotein lipid attachment sites.

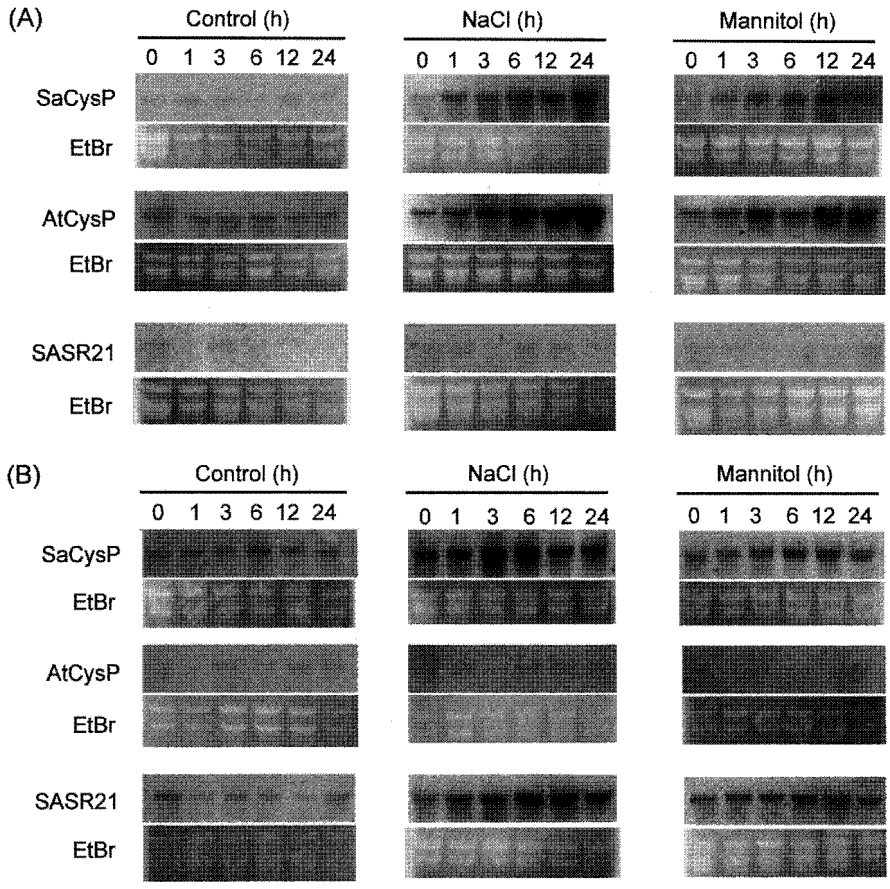

Fig. 4 Northern blot analysis of the NaCl-responsive clones hybridized to total RNA from sea aster or Arabidopsis leaves (A) and roots (B) in response to 0 mM (Control), 200 mM NaCl (NaCl), and 400 mM mannitol (Mannitol; equal to osmotic pressure for 200 mM NaCl). Leaves and roots were harvested at the indicated time intervals. An RNA blot containing 20 μg of RNA per lane was subjected to hybridization with the indicated probes. Ethidium bromide staining (EtBr) was used as a control for RNA loading. SaCysP, sea aster cysteine protease; AtCysP, Arabidopsis cysteine protease; SASR21, sea aster cysteine protease obtained by differential screening.

might function specifically in the process of adaptation to NaCl stress. The cDNA macroarray containing Arabidopsis ESTs and negative control clones (λ DNA) hybridized with ^{33}P-labeled targets derived from NaCl-treated or unstressed sea aster plants. Hybridization signals were quantified using the program package Array Vision. Global normalization and calculation of expression ratio were carried out as described in Materials and Methods. **Fig. 2** shows a scattered plot of expression ratios of NaCl-treated sample versus those of control sample. One hundred and thirty five Arabidopsis clones showed more than 2.5 fold elevation corresponding to sea aster mRNA level among the tested 5760 clones in the duplicated experiments. To isolate cDNA homologs from sea aster library, 17 clones showing higher value than the average of expression ratios were selected. One of the obtained cDNA shared 71% identity with the original cysteine protease (CysP; At4g16190) from Arabidopsis at the amino acid level (**Fig. 3**) and was named SaCysP (sea aster cysteine protease). SaCysP encoded a putative single open reading frame for 363 amino acids. The other two homologs of SaCysP in Arabidopsis showed high degrees of identity (71% with At4g39090; 70% with At2g21430). One of them is RD19A gene (At4g39090) induced by drought and salt stresses (Koizumi *et al.*, 1993). All these CysP proteins have a conserved motif of prokaryotic membrane lipoprotein lipid attachment site (**Fig. 3**). Another CysP gene named SASR21 (sea aster stress responsive gene 21) has been obtained from sea aster by differential screening method in a previous report (Takeda *et al.*, 2003). At the amino acid level, the protein encoded by SASR21 shared 87% identity with SaCysP at the C-terminus.

Transcript levels for the three CysP genes in sea aster and Arabidopsis under NaCl or osmotic stress condition were determined by Northern blot anal-

yses. Sets of both sea aster and Arabidopsis plants were hydroponically treated with 200 mM NaCl and 400 mM mannitol for various periods up to 24 h, as described in Materials and Methods. **Fig. 4** shows the time course of expression of the SaCysP, AtCysP and SASR21 genes in response to salt or osmotic stress in leaves and roots. The mRNAs corresponding to both SaCysP and AtCysP highly increased in leaves treated with NaCl and mannitol. The highest induction was observed at 24 h after salt stress and 12 h after osmotic stress in both plants. On the other hand, the mRNA corresponding to SASR21 showed no accumulation signals in leaves under normal and stressed conditions. In sea aster roots, expression of SaCysP and SASR21 mRNAs were induced by both salt and osmotic stresses. In Arabidopsis roots, expression of AtCysP was undetected under all three conditions. The genes that encode CysPs have been shown to respond to environmental stress, such as cold or desiccation (Schaffer and Fischer, 1988; Guerrero *et al.*, 1990; Koizumi *et al.*, 1993). On the other hand, Arabidopsis RD19 and RD21 mRNAs that encode CysPs were not induced by cold, heat stress or exogenous ABA (Koizumi *et al.*, 1993). These observations and our results suggest that various types of CysP genes are triggered by osmotic changes caused by high-salt or high-mannitol conditions. CysPs could be involved in the degradation of vegetative storage proteins located in vacuoles, allowing the products of such degradation to be available for the *de novo* synthesis of new proteins during stress or for osmotic adjustment, as suggested by Guerrero *et al.* (1990). SaCysP and SASR21 transcripts are differentially regulated in leaves and roots of sea aster. This result suggests that these genes may have a tissue-specific function in stress response by modulating their expression levels. The mRNAs corresponding to CysPs from sea aster were induced more strongly and the phase of induction was shifted earlier in roots than in leaves. This response system in roots may play an important role for salt tolerance of sea aster. It is hypothesized that halophytes generally use similar salt tolerance effectors and regulatory pathways that have been found in glycophytes, but that subtle differences in regulation account for large variations in tolerance or sensitivity. Although the expression profiles obtained by the hetero-probed macroarray analysis provide less information than those of normal (homo-probed) macroarry analysis, it may become a useful tool for isolation of differentially inducible genes in other plants as one of the screening methods.

References

Asamizu, E., Nakamura, Y., Sato, S., Tabata, S., 2000. A large scale analysis of cDNA in *Arabidopsis thaliana*: generation of 12,028 non-redundant expressed sequence tags from normalized and size-selected cDNA libraries. DNA Res., **7**: 175-180.

Bray, E. A., 2002. Classification of genes differentially expressed during water-deficit stress in *Arabidopsis thaliana*: an analysis using microarray and differential expression data. Ann. Bot., **89**: 803-811.

Cooke, R., Raynal, M., Laudié, M., Grellet, F., Delseny, M., Morris, P. C., Guerrier, D., Giraudat, J., Quigley, F., Clabault, G., Li, Y. F., Mache, R., Krivitzky, M., Gy, I. J. J., Kreis, M., Lecharny, A., Parmentier, Y., Marbach, J., Fleck, J., Clement, B., Philipps, G., Hervé, C., Bardet, C., Tremousaygue, D., Lescure, B., Lacomme, C., Roby, D., Jourjon, M. F., Chabrier, P., Charpenteau, J. L., Desprez, T., Amselem, J., Chiapello, H., Höfte, H., 1996. Further progress towards a catalogue of all *Arabidopsis* genes: analysis of a set of 5,000 non-redundant ESTs. Plant J., **9**: 101-124.

Gosti, F., Bertauche, N., Vartanian, N., Giraudat, J., 1995. Abscisic acid-dependent and -independent regulation of gene expression by progressive drought in *Arabidopsis thaliana*. Mol. Gen. Genet., **6**: 10-18.

Guerrero, F. D., Jones, J. T., Mullet, J. E., 1990. Turgor-responsive gene transcription and RNA levels increase rapidly when pea shoots are wilted. Sequence and expression of three inducible genes. Plant Mol. Biol., **15**: 11-26.

Iuchi, S., Yamaguchi-Shinozaki, K., Urao, T., Terao, T., Shinozaki, K., 1996. Novel drought-inducible genes in the highly drought-tolerant cowpea: cloning of cDNAs and analysis of the expression the corresponding genes. Plant Cell Physiol., **37**: 1-10.

Koizumi, M., Yamaguchi-Shinozaki, K., Tsuju, H., Shinozaki, K., 1993. Structure and expression of two genes that encode distinct drought-inducible cysteine proteinase in *Arabidopsis thaliana*. Gene, **129**: 175-182.

Kreps, J. A., Wu, Y., Chang, H. S., Zhu, T., Wang, X., Harper, J. F., 2002. Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol., **130**: 2129-2141.

Matsumura, T., Kanechi, M., Inagaki, N., Maekawa, S., 1998. The effects of salt stress on ion uptake, accumulation of compatible solutes, and leaf osmotic potential in safflower, *Chrysanthemum paludosum* and sea aster. J. Japan. Soc. Hort. Sci., **67**: 426-431 (in Japanese).

Newman, T., DeBruijn, F. J., Green, P., Keegstra, K., Kende, H., Mcintosh, L., Ohlrogge, J., Raikhel, N., Somerville, S., Thomashow, M., Retzel, E., Somerville, C., 1994. Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous *Arabidopsis* cDNA clones. Plant Physiol., **106**: 1241-1255.

Oono, Y., Seki, M., Nanjo, T., Narusaka, M., Fujita, M., Satoh, R., Satou, M., Sakurai, T., Ishida, J., Akiyama, K., Iida, K., Maruyama, K., Satoh, S., Yamaguchi-

Shinozaki, K., Shinozaki, K., 2003. Monitoring expression profiles of Arabidopsis gene expression during rehydration process after dehydration using ca 7000 full-length cDNA microarray. Plant J., **34**: 868-887.

Orcutt, D. M., Nilsen, E. T., 1996. The physiology of plants under stress. John Wiley & Sons Inc., New York.

Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press, New York.

Sasaki, Y., Asamizu, E., Shibata, D., Nakamura, Y., Kaneko, T., Awai, K., Amagai, M., Kuwata, C., Tsugane, T., Masuda, T., Shimada, H., Takamiya, K., Ohta, H., Tabata, S., 2001. Monitoring of methyl jasmonate-responsive genes in Arabidopsis by cDNA macroarray: self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. DNA Res., **8**: 153 - 161.

Schaffer, M. A., Fischer, R. L., 1988. Analysis of mRNAs that accmulate in response to low temperature identifies a CysP gene in tomato. Plant Physiol., **87**: 431-436.

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y., Shinozaki, K., 2001. Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell, **13**: 61-72.

Seki, M., Ishida, J., Narusaka, M., Fujita, M., Nanjo, T., Umezawa, T., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y., Shinozaki, K., 2002a. Monitoring the expression pattern of around 7,000 Arabidopsis genes under ABA treatments using a full-length cDNA microarray. Funct. Integr. Genomics, **2**: 282-291.

Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Taji, T., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y., Shinozaki, K., 2002b. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J., **31**: 279-292.

Shennan, C., Hunt, R., MacRobbie, E. A. C., 1987. Salt tolerance in *Aster tripolium* L. The effect of salinity on growth. Plant Cell Environ., **10**: 59-65.

Takeda, M., Uno, Y., Kanechi, M., Inagaki, N., 2003. Analyses of nine cDNAs for salt-inducible gene in the halophyte sea aster (*Aster tripolium* L.). Plant Biotechnol., **20**: 317-322.

The Arabidopsis genome Initiative, 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature, **408**: 796-815.

Ueda, A., Kanechi, M., Uno, Y., Inagaki, N., 2003. Photosynthetic limitations of a halophyte sea aster (*Aster tripolium* L) under water and NaCl stress. J. Plant Res., **116**: 65-70.

Uno, Y., Kanechi, M., Inagaki, N., Sugimoto, M., Maekawa, S., 1996a. The evaluation of salt tolerance during germination and vegetative growth of asparagus, table beet and sea aster. J. Japan. Soc. Hort. Sci., **65**: 579-585.

Uno, Y., Kanechi, M., Inagaki, N., Taki, N., Maekawa, S., 1996b. Growth and protein profile responses in the halophyte sea aster (*Aster tripolium* L.) suspension-cultured cells to salinity. J. Plant Res., **109**: 409-414.

Yamaguchi-Shinozaki, K., Koizumi, M., Urao, S., Shinozaki, K., 1992. Molecular cloning and characterization of 9 cDNAs for genes that are responsive to desiccation in *Arabidopsis thaliana*: sequence analysis of one cDNA clone that encodes a putative transmembrane channel protein. Plant Cell Physiol., **33**: 217-224.