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Expressed sequence tags from *Thellungiella halophila*, a new model to study plant salt-tolerance

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Abstract

Accumulation and analysis of expressed sequence tags (ESTs) data from halophytic plant is a relatively rapid and cheap way for discovering new genes related to salinity tolerance. We constructed a NaCl-treated cDNA library of *Thellungiella halophila* and sequenced more than 1500 randomly selected clones. By sequence analysis, 813 unique clones were identified: 549 showed homology to previously identified genes, 264 matched uncharacterized genes. All our EST data are available on the Internet. The identity between *T. halophila* and *Arabidopsis thaliana* cDNA sequences in our EST collection are 95.76% in total ESTs and 95.36% in non-redundant clones. At least eight classes of genes were related to the salt-tolerance, which accounted for about 18.89% of total sequenced ESTs. *T. halophila* that has many features similar to *Arabidopsis* can be adopted as a halophytic model for stress-tolerance research.
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Keywords: EST; Halophyte; cDNA library; *Thellungiella halophila*

1. Introduction

It is estimated that at least 20% of world's arable land and more than 40% of irrigated land is affected by salinity stress [1]. This has led the research into salt-tolerance with the aim of improving crop plants. Recently, *Arabidopsis* has emerged as an excellent model system to study plant salt-tolerance [2]; however, some novel processes or mechanisms unique to naturally stress-tolerance plants could be difficult to study with *Arabidopsis* and this could be supported by the available *Mesembryanthemum crystallinum* EST collection, which, when compared against the *Arabidopsis* genome, seems to include a number of transcripts that have no counterparts in this genome sequence. Several halophytes such as ice plant (*M. crystallinum*) and *Suaeda* species have been used extensively in physiological and molecular biological investigations; however, none of these plants is a suitable genetic model system [3]. *Thellungiella halophila* (also *Thellungiella salsuginea*) that is a close relative of *Arabidopsis* is able to withstand dramatic salinity shock up to 500 mmol/l NaCl and grow in salt far in excess of the capability of *Arabidopsis* [2]. This plant does not produce salt glands or other complex morphological alterations either before or after salt adaptation, it appears that its salt-tolerance ability is largely the result of basic biochemical and physiological mechanisms, and therefore *T. halophila* can be adopted as a halophytic model for stress-tolerance research.

Expressed sequence tags (ESTs) add information on the expressed part of the genome while representing a valuable tool in the identification of new genes, construction of linkage maps, correlation of EST map position with the position of mutant loci and the isolation of homologous genes

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from different but related species. An interesting application of EST sequencing is the studying of the gene expression pattern in response to a given environmental stimulus: the composition if a tissue mRNA population offers an overall view of the transcribed genes and, consequently is a novel tool in gene discovery and in understanding the biochemical pathways involved in physiological responses [4,5]. In this study, we collected and analyzed EST sequences from a 200 mmol/l NaCl-treated cDNA library of halophyte *T. halophila*. The genetic information obtained from this remarkable salt-tolerant crucifer will represent a key step in the discovery of genes involved in tolerance of other halophytes and glycophytes alike.

2. Materials and methods

2.1. RNA isolation and cDNA library construction

Seedlings of *T. halophila* were treated with 200 mmol/l NaCl for 48 h; aerial part tissue was collected and ground under liquid nitrogen using a mortar and pestle. Total RNA was extracted using RNAgent (Promega), poly(A^+)RNA was selected with MESSAGEMAKER kit (GIBCO BRL). First-strand cDNA synthesis used an oligo-dT linker-primer with an *Xho*I cloning site. The 5′-end of each cDNA was ligated to an adaptor with an *Eco*RI-compatible overhang. cDNA was ligated unidirectionally into the *Eco*RI and *Xho*I sites of the λ-ZAP Express vector (Stratagene), packaged in vitro, and amplified. The amplified library represents approximately 10^6 recombinants.

2.2. Sequencing

The phage library was converted to the plasmid form by mass excision according to the protocol described by Stratagene. The obtained phagemid of the library was used to infect *Escherichia coli* strain XLOLR. The bacteria were grown for 45 min and then plated at low density on medium containing Luria–Bertani broth, tetracycline (10 mg/l), and kanamycin (25 mg/l). Cultivated in 37 °C overnight, individual colonies were selected randomly for plasmid DNA purification and sequencing.

All sequencing reactions contained the standard T3 sequencing primer, and thus read into the presumed 5′-end of each cDNA. Reactions were run and analyzed on capillary automated sequencing machines (Perkin-Elmer). These machines generate two computer files for each sequencing run: a chromatogram file and a plain text file.

This study follows a random cDNA sequencing strategy instead of an approach based on subtractive hybridization for the following reasons: to obtain information on the mRNA population in salt-stressed *T. halophila* leaves; to estimate the steady state level of a transcript in this tissue under the given environmental condition; to identify multigenic families for further study of sequence conservation. A cDNA library from salt-stressed tissue was chosen because this treatment induces the transcription of a set of specific genes which was useful to survey the mRNA population under salt stress to pave the way for further studies of gene expression based on microarray technology and to identify novel genes specifically induced by the stress conditions applied.

2.3. Sequence editing

Original data analysis and assembly were performed on Macintosh operating system-based computer. The sequence text files were edited to remove leading vector and tailing, poor-quality sequence using the special program SeqClip (unpublished program). The author edited anomalous clone sequences manually after examination of their corresponding chromatogram files. Three classes of anomalous sequences were also excluded: (1) sequences without inserts at all; (2) sequences with reverse insert; and (3) sequences with incorrect adapter.

2.4. Homology comparisons

Each edited EST was translated in all six reading frames and compared with the non-redundant database at the NCBI using the BLASTX program, which compares translated nucleotide sequences with protein sequences. Default BLAST parameter values were used except for the following settings: Expect = 1, Alignments = 10, and Descriptions = 10. Sequences that returned no significant similarity were again compared using BLASTN, which compares nucleotide sequences with nucleotide sequences, with Expect = 1, Alignments = 10, and Descriptions = 10. Homologies to negative reading frames were disregarded, except in clones with inserts in the reverse orientation. Putative identifications for the ESTs were assigned based on the results of the BLAST searches and in some cases with information contained in related abstracts in PUBMED. The overall range of GenBank accession numbers are BQ091950, BQ087690–BQ087679, BQ079319–BQ079240, BQ079238–BQ079191, BQ060429–BQ060382, BQ060380–BQ060315, BQ060313–BQ060184, BM005701, BI851135–BI851126, BI851124–BI851100, BI851098–BI851088, BI698898–BI698401, BI698399–BI698394, BG734513–BG734510, BF440075–BF440060, BE758600–BE758570, BE758568–BE758531, BE727030–BE727004, BE727002–BE726998, AF499726–AF499714, BM986135–BM985461, AF499726–AF499714 and BM985461–BM986135.

3. Results

3.1. Putative identification of EST sequences

The BLAST result of each comparison was screened manually. Sequences with short base pairs, vectors or bacterial

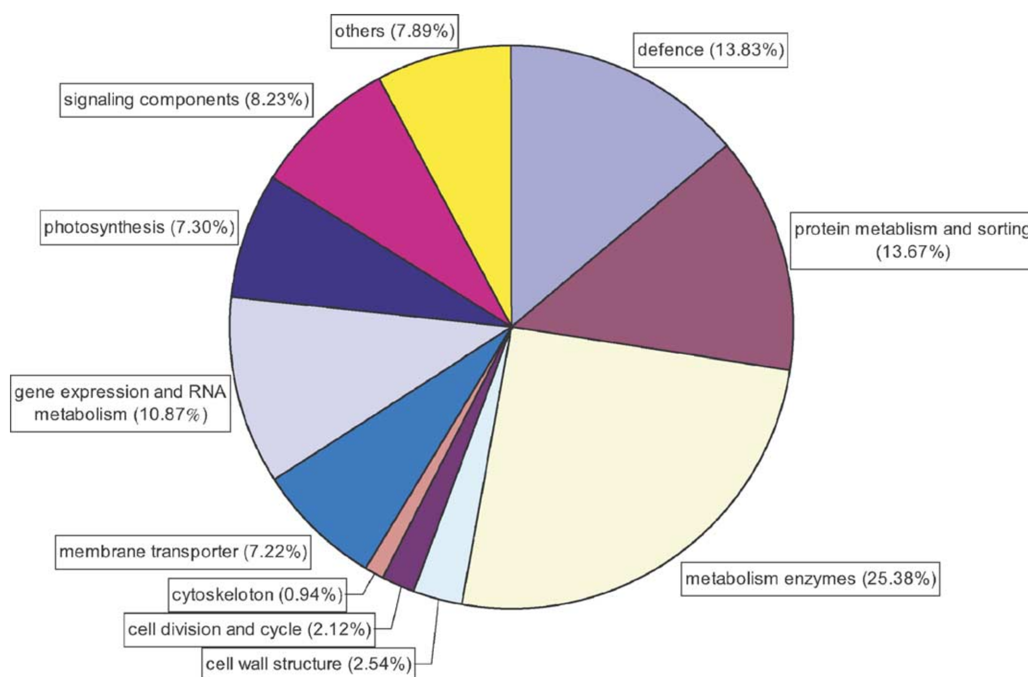

Fig. 1. Classification of 1178 identified ESTs according to putative gene function in percentage.

origin were excluded from further consideration. Finally, 1699 ESTs were retained after screening. Among them, 1178 had significant homology to previously identified genes and the distinct genes were grouped into 11 functional categories (Fig. 1).

3.2. Putative salinity stress regulated genes

During the past few years, the complex interrelationship of biochemical pathways that change during salt stress has become appreciated, although we are far from understanding this complexity, several excellent review articles are available [6,7]. According to these reviews and other related articles, we classified the genes that may be related to salt stress-tolerance of our collection into eight main groups (Table 1).

3.3. Digital Northern analysis

In addition to providing an efficient method for gene discovery, EST data sets can also provide information on gene expression. By digital Northern analysis, we found nine abundantly expressed genes (Table 2). Each kind of genes is the same gene and could be assembled into tentative unique genes (TUGs) by counting and consensus building. After sequence comparison and analysis, we got 813 unique sequences, that is, about 1/2 EST sequences in the collection are redundant. For the ESTs, 1627 out of total 1669 clones and 759 out of 813 non-redundant clones showed homolog to genes from *Arabidopsis*. (Table 3).

4. Discussion

The NaCl stress conditions applied to the halophytic plants used in the preparation of the cDNA library are effective in inducing the typical salt-tolerance process [32], and the results can therefore be considered a general survey of the mRNA population related to salt stress. Despite we got abundantly transcripts of chlorophyll *a*/*b* binding protein and RuBisCO small subunit 3b (Table 2), the percentage of photosynthesis related clones in 1178 identified ESTs was relatively low (Fig. 1). While at the same time, 13.83% of 1178 identified ESTs were related to defense, and three types of genes involved in stress responses (lipid-transfer protein, metallothionein-like protein and catalase 3) were expressed abundantly (Table 2). Maybe the shock of 200 mmol/l NaCl suppressed the process of photosynthesis and induced the stress defense in *T. halophila*. Indeed, in our studies, at least eight classes of genes were related to the salt stress-tolerance, which accounted for about 18.89% of total sequenced ESTs (Table 1). It is worth noting that an unknown protein (BM985546) whose mRNA level was up-regulated by NaCl stress (Sun, unpublished data) and light-regulated protein (BM985622) had the abundant expression in the cDNA library and the exact role of them needs further research.

An important strategy for achieving greater tolerance is to help plants to re-establish homeostasis in stressful environment. Both ionic and osmotic homeostasis must be restored. Various ion transporters are the terminal determinants of ionic homeostasis [2,6,16]. Many genes of ion transporters such as high affinity potassium transporter (BM985819), cyclic nucleotide-regulated ion channel

Table 1
Genes that may be related to salt stress-tolerance

Class of target	Example	GenBank accession number	EST clones (*n*)	Organism	Reference
Osmolyte biosynthesis genes	Trehalose-6-phosphate synthase	BI698837	2	*A. thaliana*	[6,8]
	Beta-fructosidase	BQ060245	1	*A. thaliana*	[6,8]
	Delta-1-pyrroline 5-carboxylase synthetase	BM985508	3	*A. thaliana*	[6,7,8]
	Betaine-aldehyde dehydrogenase	BQ060299	1	*A. thaliana*	[6,7]
	Galactinol synthase	BI698483	5	*A. thaliana*	[9]
Stress proteins	Cysteine proteinase RD19A	BE758597	1	*A. thaliana*	[6,10]
	Cold acclimation protein WCOR413	BI698434	2	*A. thaliana*	[6,7,10]
	Cold and ABA inducible protein kin1	BQ060250	3	*A. thaliana*	[7,10]
	Cold-regulated protein COR6.6	BQ060233	9	*A. thaliana*	[7,10]
	Low temperature and salt Responsive protein LTI6A	BI698544	3	*A. thaliana*	[7,10]
	Late embryogenesis abundant proteins-related	BM986036	3	*A. thaliana*	[10,11]
	Late embryogenesis abundant proteins	BI698820	1	*A. thaliana*	[10,11]
	Osmotin-like protein	BM985602	1	*A. thaliana*	[10,12]
	Dehydrin RAB18-like protein	BQ079203	2	*A. thaliana*	[11]
	COR47	BM985566	8	*A. thaliana*	[10]
Reactive oxygen scavengers	Peroxidase	BM985770	3	*A. thaliana*	[13]
	Neutral peroxidase c precursor	BI698577	1	*A. thaliana*	[13]
	Stromal ascorbate peroxidase	BI698822	1	*A. thaliana*	[14]
	L-Ascorbate peroxidase	BQ060229	2	*A. thaliana*	[13,14]
	Glutathione peroxidase	BM985497	8	*A. thaliana*	[13,14]
	L-Ascorbate oxidase	BM986027	1	*Brassica juncea*	[13,14]
	Ascorbate peroxidase	BQ060266	3	*A. thaliana*	[13,14]
	Monodehydroascorbate reductase	BM985962	3	*A. thaliana*	[13,14]
	Glutathione reductase, chloroplast	BQ060296	1	*B. juncea*	[13,14]
	Catalase 3	BM985979	17	*A. thaliana*	[13,14]
	Catalase 2	BE758562	3	*Raphanus sativus*	[13,14]
	Glutathione transferase	BM986050	8	*A. thaliana*	[13,14]
	2,4-D-Inducible glutathione S-transferase	BM985481	1	*A. thaliana*	[13,14]
	Type 2 peroxiredoxin related	BM985687	1	*A. thaliana*	[13,14]
	Metallothionein-like protein	BM985586	29	*A. thaliana*	[5]
	Metallothionein 2b	BQ060316	1	*A. thaliana*	[5]
	Thioredoxin H-type 3	BQ060423	15	*A. thaliana*	[15]
Transmembrane transport and ion homeostasis	Plasma membrane proton ATPase	BM985577	4	*A. thaliana*	[1,6,16]
	H^+-transporting ATPase chain E, vacuolar	AF499722	2	*A. thaliana*	[1,6,16]
	H^+-transporting ATPase	BM985886	2	*A. thaliana*	[1,6,16]
	Vacuolar ATP synthase subunit H-related	BM985669	1	*A. thaliana*	[1,6,16]
	Vacuolar ATP synthase subunit C	BI698718	1	*A. thaliana*	[1,6,16]
	High affinity potassium transporter	BM985819	1	*A. thaliana*	[1,6,16]
	Plasma membrane intrinsic protein 1B	BM986095	2	*A. thaliana*	[1,6,16]
	Plasma membrane intrinsic protein 2A	BQ079304	1	*R. sativus*	[1,6,16]
	Plasma membrane intrinsic protein 1c	BI698563	1	*A. thaliana*	[1,6,16]
	ABC transporter family protein	BM985976	3	*A. thaliana*	[1,6,16]
	Vacuolar processing enzyme (VPE) gamma	BQ060327	1	*A. thaliana*	[1,6,17]
	Voltage-dependent anion-selective channel protein hsr2	BM985465	3	*A. thaliana*	[1,6,16]
	Cyclic nucleotide-regulated ion channel	BQ079259	5	*A. thaliana*	[1,6,16]
Membrane fluidity	Non-specific lipid-transfer protein 1	BM985463	3	*A. thaliana*	[1,6,18]
	Lipid-transfer protein 6	BM985985	2	*A. thaliana*	[19]
	Non-specific lipid-transfer protein precursor-like	BM985528	27	*A. thaliana*	[19]
	Lipid-transfer protein 4	BM986040	1	*A. thaliana*	[19]
Signaling components	Polyubiquitin	BI851121	6	*A. thaliana*	[19]
	RAN2 small Ras GTP-binding nuclear protein	BM985629	4	*A. thaliana*	[20]
	GTP-binding protein-related	BM985510	1	*A. thaliana*	[21,22]
	Rac-like GTP-binding protein	BM985630	1	*A. thaliana*	[21,22]
	Ras family GTP-binding protein	BQ079319	1	*A. thaliana*	[21,22]
	Small GTP-binding protein	BI698878	1	*A. thaliana*	[21,22]
	SNF1-related protein kinase KIN10	BM985466	6	*A. thaliana*	[21,22]
	Leucine-rich repeat transmembrane protein kinase	BI698631	1	*A. thaliana*	[21,22]
	CDPK9	BM985592	3	*A. thaliana*	[21,22]

Table 1 (*Continued*)

Class of target	Example	GenBank accession number	EST clones (*n*)	Organism	Reference
	Serine/threonine-protein kinase	BQ060400	1	*A. thaliana*	[7,21,23]
	Snf1-related protein kinase KIN11	BM985842	3	*A. thaliana*	[7,21,23]
	Protein kinase family	BI698557	1	*A. thaliana*	[21,22]
	Casein kinase II beta chain CKB2	BI851129	3	*A. thaliana*	[7,21,23]
	Casein kinase	BM986001	1	*A. thaliana*	[24]
	CBL-interacting protein kinase 14	BQ079258	1	*A. thaliana*	[24]
	Serine/threonine protein kinase SOS2	BQ060265	2	*A. thaliana*	[7,21,23]
	Receptor protein kinase	BM985475	1	*A. thaliana*	[7,21,23]
	Putative protein phosphatase 2C	BM985572	7	*A. thaliana*	[21]
	Phosphatase 2C-related protein	BM985627	7	*A. thaliana*	[7,21]
	Protein phosphatase ABI1	BM985573	4	*A. thaliana*	[7,21]
	Protein phosphatase 2C	BQ079207	3	*A. thaliana*	[7,21]
	Ca/calmodulin-dependent protein kinase phosphatase	BM986018	2	*A. thaliana*	[7,21]
	Calcineurin B1 protein	BM005701	1	*A. thaliana*	[7,21,23]
	Calmodulin-binding protein	BM985941	1	*A. thaliana*	[7,21,23]
	Calmodulin	BM985799	1	*B. juncea*	[7,21,23]
Transcriptional regulators	myb family transcription factor	BM985800	6	*A. thaliana*	[21,23,25]
	14-3-3 protein GF14 omega (grf2)	BI698893	3	*A. thaliana*	[21,23]
	Homeobox-leucine zipper protein HAT9	BQ060411	3	*A. thaliana*	[23,26]
	AP2 domain transcription factor	BM985484	1	*A. thaliana*	[23]
	Zinc finger protein-related	BM985476	1	*A. thaliana*	[23,27]
	Homeobox-leucine zipper protein HAT5	BQ079221	1	*A. thaliana*	[23,27]
	Zinc finger (C3HC4-type RING finger) protein family	BM985549	1	*A. thaliana*	[23,27]
	Zinc finger protein 2, C2H2-type	BM985806	2	*A. thaliana*	[23,27]
	RING-H2 finger protein RHF1a	BM985895	1	*A. thaliana*	[23,27]
	RING zinc finger protein	BI698604	9	*A. thaliana*	[23,27]
	Heat shock protein 81-2	BQ060346	3	*A. thaliana*	[6]
	Heat shock protein hsc70-1	BM985587	8	*A. thaliana*	[6]
Metabolism	Cytosolic malate dehydrogenase	BQ060379	5	*A. thaliana*	[28]
	NADP-dependent malate dehydrogenase	BI698548	2	*A. thaliana*	[28]
	Glyceraldehyde-3-phosphate dehydrogenase-related	BM985534	7	*Sinapis alba*	[29]
	Phosphoenolpyruvate carboxykinase (ATP)-related protein	BM985903	1	*A. thaliana*	[30]
	Putative aldehyde dehydrogenase	BE758558	1	*A. thaliana*	[31]
Total			321		

(BQ079259), ABC transporter family protein (BM985976) and voltage-dependent anion-selective channel protein hsr2 (BM985465) were included in our dbEST. Secondary active transport and electrochemical flux across the plasma membrane and tonoplast are driven by the H^+-pumps such as H^+-ATPase and H^+-pyrophosphatase and several ESTs related to plasma membrane H^+-ATPase (BM985577), tonoplast H^+-ATPase (AF499722, BM985669, BI698718) also existed in our dbEST.

As well as maintaining ionic homeostasis in the cell cytosol, plants under salt stress also need to establish water or osmotic homeostasis. The accumulation of osmolytes such as sugars, sugar alcohols and complex sugars is believed to facilitate "osmotic adjustment", by which the

Table 2
Most abundant mRNAs

GenBank accession number	Putative identification	No. of ESTs	Percentage of total ESTs
BM985586	Metallothionein-like protein	29	1.71
BM985528	Non-specific lipid-transfer protein precursor-like	27	1.59
BM985993	Photosystem II type I chlorophyll *a*/*b* binding protein	20	1.18
BM985979	Catalase 3	17	1.00
BI698705	Unknown protein	17	1.00
BM985622	Light-regulated protein	16	0.94
BM985552	RuBisCO small subunit 3b	15	0.94
BQ060423	Thioredoxin-H	15	0.88
BE727012	dTDP-D-glucose 4,6-dehydratase	14	0.82

Table 3
Summary of EST clones

Category	No. of clones	
	Total EST clones	Non-redundant clones
Homology to known transcripts	1178 (69.33)	549 (67.53)
A. thaliana	1123 (66.10)	512 (62.98)
Brassica nupus	14 (0.82)	8 (0.98)
B. juncea	6 (0.35)	5 (0.61)
Others	35 (2.06)	24 (2.95)
Homology to unclassified proteins	521 (30.67)	264 (32.47)
A. thaliana	504 (29.66)	247 (30.38)
Others	17 (1.00)	17 (2.09)
Total	1699 (100)	813 (100)

The values given in parentheses are in percentage.

internal osmotic potential is lowered [6]. Many ESTs related to sugar synthesis were included in our dbEST, among them trehalose-6-phosphate synthase (BI698837) was the key enzyme in trehalose synthesis. In the case of transgenic plants that produce trehalose, trehalose itself might well be a signaling molecular for growth control as well as for stress-tolerance. Pyrroline-5-carboxylate synthetase (BM985508)—a bifunctional enzyme for proline biosynthesis that was induced by dehydration and high-salinity stress, galactinol synthase (BI698483)—catalyses the first step in the biosynthesis of RFOs which are involved in tolerance to drought, high-salinity and cold stresses and betaine aldehyde dehydrogenase (BQ060299) that was involved in the synthesis of glycine betaine were also included in our dbEST. In addition, water channel proteins such as plasma membrane intrinsic protein 1B (BM986095), 2A (BQ079304) and 1c (BI698563) might be involved in controlling the speed of water flux across cellular membranes under salt stress to help to sustain osmotic homeostasis [2]. About 2.30% of total mRNA populations in so small a dbEST library were related to re-establish homeostasis, which indicated the importance of homeostasis in salt-tolerance of *T. halophila*.

Under salt stress, an important cause of damage might be reactive oxygen species (ROS) generated by salt stress. ROS scavenging as an important component of abiotic responses is documented by transgenic plants overexpressing enzymes involved in oxidative protection, such as glutathione peroxidase, superoxide dismutase, ascorbate peroxidases, bacterial catalase and glutathione reductases increased plant salt-tolerance [2,6]. We found 5.77% EST clones in our dbEST related to ROS scavenging such as L-ascorbate peroxidase (BQ060229), catalase 3 (BM985979) and glutathione peroxidase (BM985497). So many transcripts of ROS scavenging enzymes appeared in this small dbEST library indicated that *T. halophila* owned an effective pathway to detoxification under salinity environment.

Under stress condition, many stress proteins were also produced to improve salt-tolerance (Table 1). Many cold induced proteins were also induced in this salt-stressed cDNA library, which demonstrated the feasibility of gene transcription related to stress-tolerance.

Salt-tolerance also involves changes in the levels and composition of fatty acids of the major glycerolipids in roots and leaves of a wide range of plants. Changes in the level of fatty acid saturation/unsaturation have been reported as a response to salt stress. About 1.94% lipid-transfer proteins (Table 1) were collected in our ESTs library, which indicated active lipid metabolism or/and salinity defense during salt stress.

Halophytes might also have evolved distinct stress-recognition of signaling pathway, and regulatory controls that confer stress protection (genomic approaches to stress-tolerance). Environmental signals are thought to be first perceived by specific receptors that, upon activation, will initiate (or suppress) a cascade to transmit the signal intracellularly and in many cases, activate nuclear transcription factors to induce the expression of specific sets of genes [21,23]. Although none of the receptors for salinity in plants is determined to certainty, current knowledge indicates that receptor-like protein kinases (BM985475 in our dbEST), two-component histidine kinases, as well as G-protein-associated receptors may represent the potential sensors of these signals.

A family of protein kinases unique to plants, the calcium-dependent protein kinases (CDPKs), is also involved in stress responses [7,21]. The overexpression of the rice CDPK7 gene in rice recently has been shown to increase tolerance to low temperature, drought, and high salt. Three transcripts of calcium-dependent protein kinase 9 (BM985592) existed in our dbEST, which had 83% identify with *Arabidopsis*.

In a stress-signaling cascade, inactivation of phosphoproteins is usually accomplished by dephosphorylation. There are four major subgroups of protein phosphatases: PP1, PP2A, PP2B (calcineurin) and PP2C. Nine clones of PP2C were included in our dbEST, suggesting that the alignment was not due to chance. The characterization of a yeast mutant defective in calcineurin B (CNB, PP2B) underscores the crucial role of this protein phosphatase in salt-tolerance [6,7,21]. By functional complementation, plant transformation or expression studies, several homologous plant proteins are implicated to function in salt-tolerance in a similar way to the yeast CNB. Identification of the salt overly sensitive 3 (*SOS3*) gene product as related to CNB provided compelling evidence to support the role of calcineurin-like protein in plant salt-tolerance. However, SOS3 does not seem to function through a protein phosphatase. Rather, SOS3 interacts with and activates a serine/threonine protein kinase encoded by *SOS2*. The SOS3–SOS2 protein kinase complex is becoming a well-studied module that functions in plant salt-tolerance [7,21,23]. We found transcripts of calmodulin (BM985799), calcineurin B1 protein

(BM005701) and serine/threonine protein kinase SOS2 (BQ060265). More research including yeast two-hybrid should be done to survey if there is a similar pathway for ionic homeostasis under salt stress in *T. halophila*.

Expression of regulatory genes that control several steps in a pathway or in related pathways (i.e. a regulon) might provide an alternative to introducing pathway genes one at a time. The basic principle is to manipulate the expression of a regulatory gene (e.g. a specific transcription factor), which in turn alters the expression of the entire regulon. Ectopic expression of the dehydration-responsive element-binding protein under the control of the stress-inducible rd29A promoter resulted in the production of *Arabidopsis* plants that had improved tolerance of freezing, drought and high-salinity stress [33], which demonstrated the feasibility of this approach in a plant stress context. Several transcription factors, such as homeobox-leucine zipper protein (BQ079221) and 14-3-3 protein (BI698893) also exist in the EST collection, and may be, therefore, an attractive candidate for regulon engineering.

In this dbEST, we got at least 101 ESTs related to salt-tolerance signaling components or transcription regulators. So small dbEST library with so many signaling components indicated an effective signaling cascade in *T. halophila* under salt stress.

It is now hypothesized that halophytes use salt-tolerance effectors and regulatory pathways very similar to those in glycophytes and that subtle differences in their regulation can account for large variations in salt sensitivity [2,3]. Many investigators began to realize that to directly test this hypothesis, genes responsible for tolerance mechanisms operating in halophytes must be discovered through functional genetic analysis and the novelty of their functions (compared with their glycophyte versions) subsequently determined. This would require the use of a halophytic model system that provides experimental expediency similar to that of *Arabidopsis*. That is, a model would be needed that had: (a) desirable life history traits, i.e. small size, short life cycle, self-pollination, and high seed number; and (b) favorable genetic traits such as self-fertilization, a small genome, efficient transformation, and mutagenesis. At present, *T. halophila*, which is able to withstand dramatic salinity shock up to 500 mmol/l NaCl, meets all of these criteria. Among our EST collection, 1627 out of total 1669 clones and 759 out of 813 non-redundant clones showed homology to genes from *Arabidopsis*. That is to say the identity between *T. halophila* and *Arabidopsis thaliana* cDNA sequences in our EST collection are 95.76% in total ESTs and 95.36% in non-redundant clones. Some clones, such as BM985898 has no *Arabidopsis* homolog in the GenBank with BLASTX and BLASTN analysis and the possibility of unique transcript in halophyte *T. halophila* need further study. Perhaps with more EST clones being sequenced, we could get more special transcripts. *T. halophila*, which is a close relative of *Arabidopsis*, could be a model system in discovering salt-tolerance determinants and pathways operating in the halophyte with molecular genetics techniques that characterize *Arabidopsis*.

We are pursuing several strategies, such as map-based cloning, EMS/T-DNA insertion mutation and RNAi to identify highly specialized genes that control the extreme salt-tolerance of *T. halophila* with the information gotten from EST collection. Despite we got many genes related to salt-tolerance in so small a EST library, the value of these sequences will become fully appreciated only when the next step in our project is included, microarray analysis of the cDNAs. Perhaps by analyzing microarray-derived gene expression data, we can cluster the transcript behavior into different groups according to induction or repression identifies patterns that might relate to developmental contexts or physiological pathways. Functionally unknown gene can be grouped with known transcripts with similar expression patterns, possibly due to functional relatedness, which can then be tested.

The EST data from *T. halophila* cDNA library described here can initially be used to create codon-usage tables and other data tables to assist in the establishment of *T. halophila* as a model system for molecular genetic studies of plant salinity tolerance. Eventually, genetic information obtained from this remarkable salt-tolerant crucifer will represent a key step in the discovery of genes involved in tolerance of other halophytes and glycophytes alike.

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