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Cloning and expression analysis of salt responsive gene from *Kandelia candel*

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Abstract

Identification of gene expression patterns in mangroves grown under salinity will help to reveal the molecular mechanisms of salt tolerance. Here, 10 cDNAs of genes were isolated from *Kandelia candel* and identified by representational difference analysis of cDNA (cDNA RDA) under different NaCl concentrations. Of five genes expressed preferentially under salt condition, two were unknown, three were two kinds of low molecular mass heat-shock proteins (sHSPs) and ADP-ribosylation factor, respectively. The expressions of other five genes were repressed under NaCl stress, two encoded cyclophilins, three were tonoplast intrinsic protein, early light-induced protein and 60S ribosomal protein, respectively.

Additional key words: mangroves, representational difference analysis of cDNA, salt tolerance.

Introduction

Soil salinity is an important agricultural problem, and the identification of the salt stress responsive genes or salt tolerance genes is essential for breeding programs. Salt tolerance, a complex, multifactorial, and multigenic process, has been considered to be a quantitative trait (Winicov 1998). Recently, more attention has been focused on the products of structural genes (transport proteins, ion channels, enzymes of solute synthesis) with some attention to products that may have regulatory roles (Anthony 1998).

Mangroves are wood plant communities, which grow on the intertial zone of tropical and subtropical coast. Mangroves are divided into two distinct groups based on their salt management strategies. One is "secretors" which have salt glands or salt hairs and the other is "non-secretors" lacking such morphological features. *Kandelia candel* is one of the dominant species of mangroves along the Chinese coast (Li and Lee 1997), which belongs to the latter. Thus, *K. candel* is a perfect material to clarify the molecular mechanism of salt tolerance in mangrove plants. The developmental biology, population genetic structure, the correlation of viviparous reproduction with abscisic acid (ABA), and some physiological characters of *K. candel* had been recognized (Mei *et al.* 1998, Elizabeth *et al.* 1998, Lin 1999). However, patterns of gene expression of *K. candel* under various growth conditions have not yet been reported. Here, the gene expression of *K. candel* under two different NaCl concentrations were investigated, these results may help to understand the molecular mechanisms of salt tolerance.

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Abbreviations: ABA - abscisic acid; RDA - representational difference analysis.
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Materials and methods

Plants: Viviparous seeds of *Kandelia candel* Druce (*Rhizophoraceae*) were collected from Hainan province, China, and grown in pot with sand and nutrition solution. The plants were irrigated every 3 d with water. After 2 months growth, some young plants were transferred to a nutrition solution with 3 % NaCl (m/v) for 12 h (NaCl stress), some still planted with nutrition solution (control), and then total RNA was extracted from root system.

Oligonucleotides: Sequences of oligonucleotides used in representational difference analysis of cDNA (cDNA RDA) were as follows:
R-Bam-24: 5'-AGCACTCTCCAGCCTCTCACCGAG-3'
R-Bam-12: 5'-GATCCTCGGTGA-3'
J-Bam-24: 5'-ACCGACGTCGACTATCCATGAACG-3'
J-Bam-12: 5'-GATCCGTTCATG-3'

Isolation of total RNA and cDNA synthesis: Total RNA was isolated by the method of CTAB (Fang *et al.* 1998). Roots were ground in liquid nitrogen, and incubated at 65 °C for 15 min in 20 cm^{-3} extraction buffer (20 % CTAB, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 4 % 2-mercaptoethanol). An equal volume of chloroform:isoamyl-alcohol (24:1, v/v) was added to the suspension, and centrifuged at 11 500 *g* for 15 min. The extraction and centrifugation steps were repeated once. RNA was selectively precipitated and purified by using 2 M LiCl precipitation at -20 °C overnight, and recovered by centrifugation at 11 500 *g* for 1.5 h. The RNA pellet was washed twice in 20 cm^{-3} 5 % ethanol and centrifuged at 11 500 *g* for 20 min. Then the pellet was resuspended in 0.2 cm^{-3} diethyl pyrocarbonate (DEPC)-treated water. cDNA synthesis was performed by random priming of 1 μg total RNA using SMART™ PCR cDNA synthesis kit (*Clontech*, USA).

cDNA representational difference analysis: The basic method used was similar to that as described by Hubank and Schatz (1994), with some modifications (Huang *et al.* 2002). Two cDNA populations were digested with DpnII (New England Biolabs) and ligated to the R-Bam-12/24 and J-Bam-12/24 adapter, separately. Amplicons for "tester" (including target genes) and "driver" (no target genes) were generated with PCR amplication for 20 cycles (95 °C for 15 s, 72 °C for 2 min). Four rounds of subtractive hybridization were used. The tester: driver ratios were 1:400 [differential product (DP1)], 1:4000 (DP2), 1:4 000 000 (DP3), 1:400 000 000 (DP4). Subtractive hybridization was performed in 0.002 cm^3 reactions (30 mM HEPPS, 3 mM EDTA, 1 M NaCl, 10 % PEG 8 000, pH 8.0) at 67 °C for 20 h. After each round of hybridization, products were digested with 20 U of mung bean nuclease (*New England Biolabs*, USA) in a volume of 0.04 cm^3, and amplified by PCR to generate the difference product.

Cloning of differential products and arraying of cloned RDA products: The differential products from the fourth round were cloned into the pGEM-T vector (*Promega*, USA) and the ligation mixture was transformed into competent DH5 *Escherichia coli* cells. The library was plated onto ampicilin-containing agar plate and bacterial were grown at 37 °C until clones were visible. Individual clones were picked out and PCR amplified directly using corresponding RDA primer in 0.02 cm^{-3} PCR reactions containing standard PCR buffer, 200 mol m^{-3} dNTP, 0.5 U Hotstar Taq polymerase (*Qiagen*, The Netherlands) and 0.1 μg primer. Thermal cycling conditions consisted of. 95 °C for 15 min, followed by 35 cycles of 95 °C for 15 s, 72 °C for 2 min, with a final extension at 72 °C for 10 min. Nine PCR amplification products were picked. random and examined by agarose gel electrophoresis and ethidium bromide staining. A single band was detected in all nine products. 0.002 cm^3 of each PCR product was deposited in duplicate onto Hybond N^+ nylon membranes (*Amersham Pharmacia Biotech*, USA). Filters were denatured, hybridized with equivalent amounts of ^{32}P-labelled double standard cDNA of driver and tester (adaptors were removed) in 100 μg cm^{-3} sheared and denatured salmon sperm DNA, 1 % SDS, 5×SSPE, 5×Denhards solution for 20 h at 68 °C. Filters were washed under stringent conditions (2×SSC, 0.1 % SDS at room temperature twice followed by two 15 min washes in 0.2×SSC, 0.1 % SDS and 0.1×SSC, 0.1 % SDS at 65 °C) and exposed to X-ray film with a high speed intensifying screen at -80 °C for 1 - 3 d.

Northern analysis: Total RNA was electrophoresed in denaturinformaldehyde agarose gels. Following overnight capillary transfer, Hybond H^+ nylon membranes was fixed at 80 °C for 2 h. Probes were generated by PCR amplification of the insert of interest, labeled with the random primed DNA labeling Kit (*Promega*). Hybridization and washing conditions were carried out as described above. Ethidium bromide stained rRNAs were used as loading control.

DNA sequence and analysis: Differential products were sequenced using M13 reverse and forward primers. Resulting sequences were compared to GenBank using the BLAST program (Dennis *et al.* 2000).

Results

Isolation of salt responsive gene cDNA clone: Identification of differentially expressed genes using cDNA RDA requires the samples of a population grown under the condition of interest and a population grown in exactly the same way but differing in the condition of interest. To isolate the genes in *K. candel* that are preferentially expressed under salinity, we used cDNA from the NaCl-treated plant as the tester and control plant as the driver, which we termed forward subtraction. To isolate the genes that were down regulated by NaCl, we performed a reversed subtraction in which the control plant served as the tester and the NaCl-treated plant as the driver. The schedule of RDA was showed in Fig. 1.

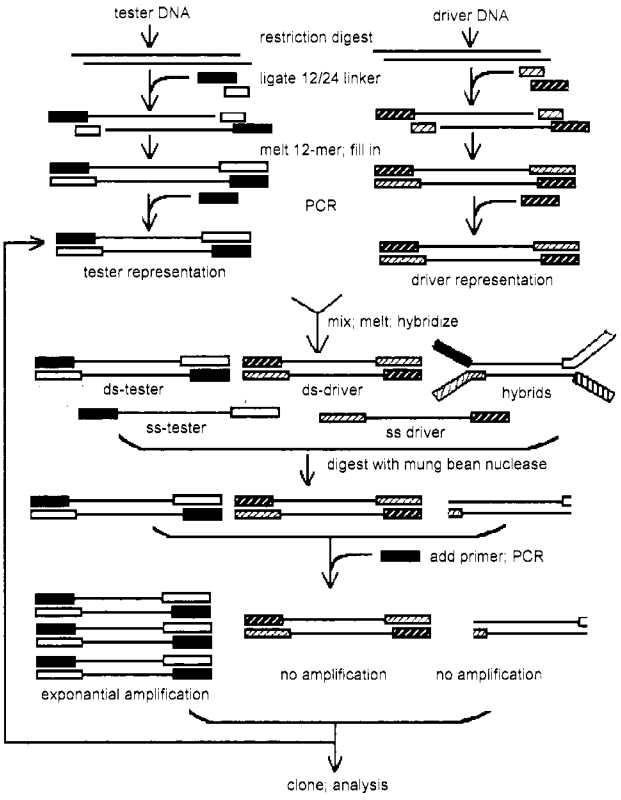

Fig. 1. The scheme of modified cDNA RDA.

According to the hybridization ratio suggested by Hubank and Schatz (1994), after an initial round of subtraction, we obtained three major distinct bands, which were enriched further in next two rounds of hybridization. To determine whether these fragments were differentially expressed, we recovered one band and labeled it with ^{32}P-dCTP. Southern analysis showed that probe hybridized strongly with both driver and tester cDNA, demonstrating that the differential band was false positives. To avoid the reappearance of these false positives, we performed the second hybridization by increasing the ratio of tester/driver. In the second cDNA RDA, three major bands disappeared gradually in four rounds of subtractive hybridization, and some novel bands enriched to be visible in a smear background (Fig. 2).

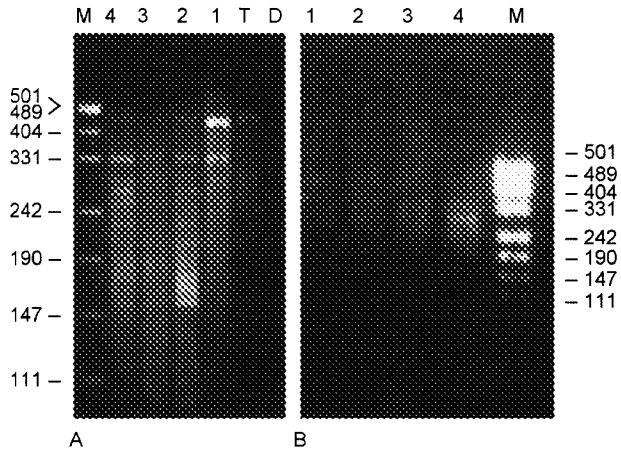

Fig. 2. Electrophoresis of difference products obtained by RDA. Different products of forward subtraction were run on a 4 % FMC agarose gel (*A*). Different products of reverse subtraction were run on a 3 % agarose gel (*B*). D - driver amplicons; T - tester amplicons; M - marker; 1 to 4 - different products (DP1 - DP4).

After purification by Qiaquick PCR purification kit, differential products (DP4) were shotgun cloned into the pGEM-T vector. Individual clones were picked into 96 well microtiter plates and inserts were amplified with RDA primer. RDA products were arrayed in duplicate on Hybond H^+ nylon membrane and co-hybridized with ^{32}P-dCTP labeled driver and tester initial amplicons. After stringent washing and exposing, hybridization signals were compared directly between two hybridizations. At a differential expression ratio of two, 41 cDNA clones differentially expressed in forward subtraction. When the differential ratio increased to a factor of five or more, seven cDNA clones were considered differentially expressed. At a differential expression ratio of two, 24 clones were differentially expressed in reverse subtraction.

Sequence and expression analysis of salt responsive gene cDNA: The expression pattern of individual clone was investigated by Northern blot analyses. The cDNA inserts were isolated from the sequenced clones and used as hybridization probes on blots containing salt-treated and control total RNA. Of the tested 18 genes, one showed no detectable expression in Northern blots. This most likely indicates that these fragments exist at low abundance in both samples. Seven clones detected strong

signal with no differences in expression between two samples, thus, they were considered background (data not shown). The other 10 clones showed differential expression in two RNA populations (GenBank accession number: AF521137-AF521144, AF21146, AF525702). Among these 10 clones, Northern analysis (Fig. 3) showed that five were salt-induced genes, which were termed as SIGKC1-5 (salt-induced gene in *K. candel*), respectively; five were salt-repressed genes, which were termed as SRGKC1-5 (salt-repressed gene in *K. candel*), respectively. The transcription level of SIGKC5 increased a little (data not shown), SIGKC1-4 increased 2 - 5 fold in salt treated *K. candel* seedlings compared to control. SRGKC1 and SRGKC2 displayed suppressed expression under salt stress in different varieties (Figs. 3 and 4).

cDNAs of SIGKC1-5 and SRGKC1-5 were sequenced, and the sequences were compared with the sequences registered in the databases. Both SIGKC1 and SIGKC 2 were identical in the range of 61 - 71 % to cytosolic low molecular mass heat-shock proteins (sHSPs) from tobacco and tomato, and encoded a sHSP, respectively. SIGKC3 showed 93 % identity to ADP-ribosylation factor (ARF) from *Arabidopsis thaliana*, SIGKC4 and SIGKC5 showed no significant similarity with known protein (Table 1). Of five sequences identified from the reverse subtraction, SRGKC1 was 91 % identical to the gamma tonoplast intrinsic protein from *Pyrus communis* over a stretch of 61 amino acids. SRGKC2 displayed 90 % sequence identity over a stretch of 84 amino acids to the cyclophilin from *Euphorbia esula,* and SRGKC3 was 93 % sequence identity over a stretch of 47 amino acids to the hypothetical protein vcCyP from *Vicia faba.* SRGKC4 showed 67 % identity to early light-induced protein from *Arabidopsis thaliana*, SRGKC5 was 91 % identical to 60s ribosomal protein L13a from *Arabidopsis thaliana* (Table 2).

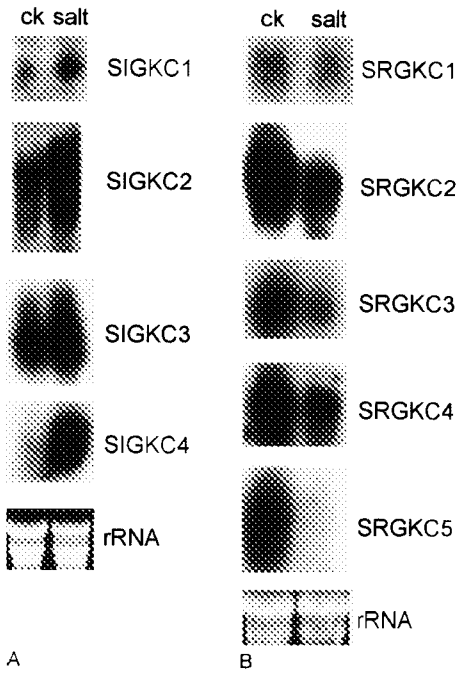

Fig. 3. Northern analysis of salt responsive genes. Differential fragments were used as probes in Northern blots to determine the expression levels in salt-treated (salt) and control (ck) samples. *A* - four clones preferentially expressed in the salt-treated sample; *B* - five clones displayed suppressed expression in the salt-treated sample.

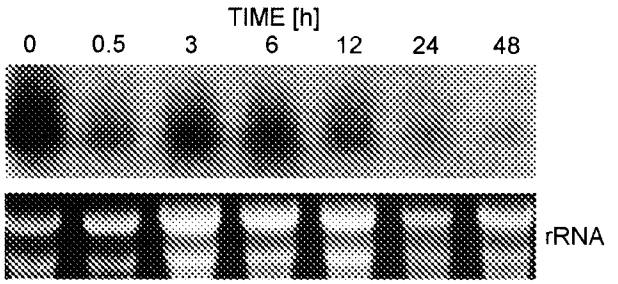

Fig. 4. Time course analyses of SRGKC1 expression at different time after 3 % NaCl treatment. Total RNA was isolated at 0, 0.5, 3, 6, 12, 24, 48 h after 3 % NaCl treatment. Northern blot hybridization with the radioactively labeled SRGKC1 at the indicated time points.

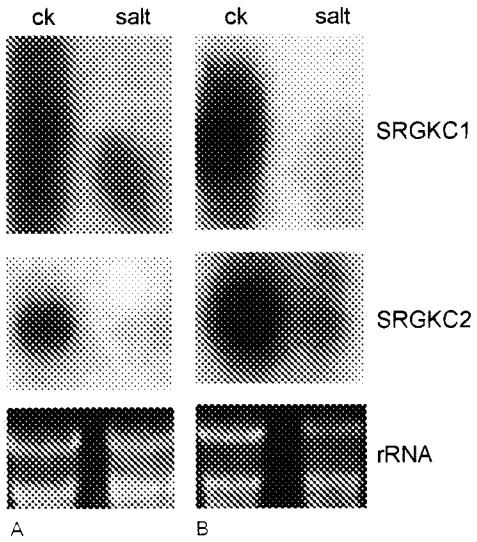

Fig. 5. Analysis of SRGKC1 and SRGKC2 gene expression in other species. Total RNA was used for Northern blot hybridization with the radioactively labeled SRGKC1 and SRGKC2. *A* and *B* - total RNA isolated from *Rhizophora apoculata* and *Ceriops tagal* after salt treatment for 12 h.

Table 1. Sequence identity of clones up-regulated.

Clone	Homology protein	Length [bp]	GenBank accession No.
SIGKC1	cytosolic class I low molecular mass HSP	399	AJ009880
SIGKC2	cytosolic class II low molecular mass HSP	284	U72396
SIGKC3	ADP-ribosylation factor (ARF)	144	AF022389
SIGKC4	Unknown protein	83	
SIGKC5	Unknown protein	237	

Table 2. Sequence identity of clones down-regulated.

Clone	Homology protein	Length [bp]	GenBank accession No.
SIGKC1	gamma tonoplast intrinsic protein	175	AB048248
SIGKC2	cyclophilin	282	X74403.1
SIGKC3	hypothetical protein vcCyP	160	T50770
SIGKC4	early light inducible protein like protein	224	U89014
SIGKC5	probable 60s ribosomal protein	236	AF051212

Discussion

According to the hybridization ratio suggested by Hubank and Schatz (1994), there got high false positives. cDNA RDA requires the production of high quality representative cDNA. mRNA accounts for at most 5 % of total RNA, the rest may be rRNA and tRNA. Utilizing total RNA as a template to synthesize cDNA can not avoid a fraction of cDNA derived from rRNA, which will interfere with the cDNA RDA procedure, particularly during the subtraction and PCR enrichment steps. Even if synthesizing cDNA with mRNA directly, some high-abundance mRNA sequences will be enriched preferentially, these high-abundance sequences might suppress the enrichment of the low abundance fragments (Hubank and Schatz 1994, Bowler *et al.* 1999). Thus, adjusting the hybridization ratio properly may be beneficial for eliminating the interference of non-target fragments.

Mangroves grow under salinity and flooding. Up to now, little is known about the genetic mechanisms of mangroves for salt tolerance. However, there was obvious improvement in the salt tolerance among the transformed plants by introduced total DNA of *Rhizophora apoculata* into *Capsicum annuum* (Lin *et al.* 2001), indicated that salt resistance gene existed in mangroves genome. In this experiment, ten salt responsive genes were identified, such as sHSP, ADP-ribosylation factor, tonoplast intrinsic protein, cyclophilins as well as early light-induced protein-like, suggested these stress proteins may be involved in salt tolerance mechanisms of mangroves.

Small Mr heat shock proteins (sHSPs) are a diverse group of heat inducible proteins that are conserved in prokaryotes and eukaryotes and are especially abundant in plants. Two conserved classes, designed class I (HSP18.1) and class II (HSP17.7) proteins, are located in the cytosol (Garrett *et al.* 1995). The sequences of the SIGKC1, 2 each represent different membranes of sHSPs subfamilies. The predicted amino acid sequence of SICKC1 is 72 % identical to 17.6 kD class I small heat shock protein from *Lycopersicon esculentum* (GenBank accession number AF123257), and is similar (66 % identical) to 17.7 kDa heat shock protein from *sunflower* (GenBank accession number S71566). The predicted protein sequence of SIGKC2 is 73 % identical to cytosolic class II low molecular mass heat shock protein from *Prunus dulcis* (GenBank accession number AF159562). Recent *in vitro* data indicated that sHSPs might act as molecular chaperones to prevent thermal aggregation of proteins by binding non-native intermediates (Collada *et al.* 1997, Garrett *et al.* 1997). Blast searches and expression analysis implied that two sHSPs might have roles in preventing irreversible heat-inactivation of other proteins. SIGKC3 is 93 % identical to a putative ADP-ribosylation factor from *Arabidopsis thaliana* in the range of 32 amino acids (GenBank accession number NM100939). The previous study indicated that ADP-ribosylation factor (ARF) is a ubiquitous, highly conserved 21 kDa GTP-binding protein, first identified in animal cells as the cofactor required for the *in vitro* ADP-ribosylation of the stimulatory regulatory subunit of adenylate cyclase (Stearns *et al.* 1990). The ADP-ribosylation factor proteins comprise a group of five Ras-related GTPases that are thought to function as regulators of membrane traffic (Rita 2000). The accumulation of ARF mRNA may provide a useful osmotic equilibrium mechanism by which the *K. candel* plant can resist salt damage.

Aquaporins are members of the MIP (membrane intrinsic protein) family and include TIP (tonoplast intrinsic protein) and PIP (plasma membrane intrinsic protein) membranes. The discovery of water-channel proteins in membranes of plant cells reveals potential new mechanisms that may be used by plants to control water transport and osmotic adjustment (Maurel 1997). Aquaporins have two postulated functions: a role of TIPs in intracellular osmotic equilibration and a role of PIPs in regulation of the transcellular water transport (François *et al.* 1998). Studies of the aquaporin gene family are most complete for *A. thaliana* (Weig *et al.* 1997) and *M. crystallinum* (Tyerman *et al.* 1999). The deduced amino acid sequence of SRGKC1 is 88 % identical to that of a salt-stress TIP from *A. thaliana* (GenBank accession number AF004393) and 86 % identical to TIP from *M. crystallinum* (GenBank accession number U43291). Salt may result in large and rapid changes in extracellular water potential and serious disturbance to the cytoplasm. In order to compensate for this imbalance, the relative contribution of water channels to flow across the root could thus vary (Tyerman 1999). Leaves of *Avicennia germinans* featured low internal conductance to water transport under high salinity (Sobrado 2001). The expression of SRGKC1 was repressed by salt. *K. candel* is a species that is native to intertial zone of tropical and subtropical coast and is well-adapted to salt conditions. The coordinate down-regulation of aquaporins in this plant may decrease membrane water permeability and thus increase the cellular water conservation during periods of salt stress. Our results are consistent with postulated roles for tonoplast water channels in regulating the hydraulic permeability of the vacuolar membranes and in adjusting the water homeostasis of the protoplasm under various physiological conditions (François *et al.* 1998).

The predicted amino acid sequences of SRGKC2 and SRGKC3 are most similar to cyclophilin from *Euphorbia esula* and *Vicia faba*, respectively (GenBank accession number AF242312, T50770). Cyclophilins are a group of highly conversed proteins present in all organisms. Initially CyP was identified as a high affinity binding protein for the immunosuppressive drug cyclosporin A (CsA) (Handschumacher *et al.* 1984). In addition, CyPs have been found to have peptidyl-prolyl *cis-trans* isomerase (rotamase) activity, accelerating the correct folding of proteins (Freskgard *et al.* 1992). When the plants were exposed to different stress, such as low temperature, ABA, drought, wounding, *etc.*, the amount of the cyclophilin mRNA was markedly increased suggesting that cyclophilins play a role in several responses in plants (Leonardo *et al.* 1998). However, in our experiment, the pattern of the cyclophilin gene expression in response to salt was different from that previously reported. In order to validate this case, Northern blot analyses of clone SRGKC2 in other two species of mangroves were performed, and results conformed to in *K. candel*. An increased content of cyclophilin might be necessary to assist in rapid protein synthesis, this general function might be broadened to the stimulation of folding of denatured proteins in stress tissue (Anne *et al.* 2000). Cyclophilin expression reduced in mangroves under salt stress was special phenomena, the role of cyclophilin was unclear, and the relationship between salt stress and the down expression in *K. candel* requires further investigation. In addition, the decreasing of early light-induced protein and 60S ribosomal protein mRNAs level under salt stress might reduce energy consumption in roots and benefit for plant to live through adverse circumstance conditions.

K. candel might share common salt-tolerant mechanisms with other plants including a role for TIP in intracellular osmotic equilibration, sHSPs as chaperon in protein protection. Down regulations of cyclophilin and ELIP were related to salt stress, but whose roles of these special varieties and unknown proteins played in salt-tolerance remains to be elucidated, which might help to understand the molecular mechanisms of salt tolerance of *K. candel*.

References

Anne, N.S., Sabine, K., Gerd, K., Gunter, F., Bernd, F.: Oxygen stress increases prolyl cus/trans isomerase activity and expression of cyclophilin 18 in rabbit blastocysts. - Biol. Reproduct. **62**: 1-7, 2000.

Anthony, Y.: Molecular biology of salt tolerance in the context of whole plant physiology. J. exp. Bot. 49: 915-929, 1998.

Bowler, L.D., Hubank, M., Spratt, B.G.: Representational difference analysis of cDNA for the detection of differential gene expression in bacteria: development using a model of iron-regulated gene expression in *Neisseria meningitidis*. - Microbiology **145**: 3529-3537, 1999.

Collada, C., Gomez, L., Casado, R., Aragoncillo, C.: Purification and *in vitro* chaperon activity of a class I small heat-shock protein abundant on recalcitrant chestnut seed. - Plant Physiol. **115**: 71-77, 1997.

Dennis, A.B., Ilene, K.M., Lipman, D.J., Ostell, J., Rapp, B.A., Wheeler, D.L.: GenBank. - Nucleic Acids Res. **28**: 15-18, 2000.

Elizabeth, J., Farnsworth, J.M.F.: Reductions in abscisic acid are linked with viviparous reproduction in mangroves. - Amer. J. Bot. **85**: 760-769, 1998.

Fang, X.D., Wu, D.G., Lin, Q.F., Li, G.Y.: [A suitable method for RNA extraction of halophytes.] - Nat. Sci. J. Hainan Univ. **16**: 311-313, 1998. [In Chinese.]

Francois, B., Chaumont, F., Chrispeels, M.J.: High expression of the tonoplast aquaporin ZmTIPI in epidermal and conducting tissues of maize. - Plant Physiol. **117**: 1153-1163, 1998.

Freskgard, P.-O., Bergenhem, N., Jonsson, B.H., Svensson, M., Carlsson, U.: Isomerase and chaperon activity of prolylisomerase in the folding of carbonic anhydrase. - Science **258**: 466-468, 1992.

Garrett, J.L., Pokala, N., Vierling, E.: Structure and *in vitro* molecular chaperon activity of cytosolic small heat shock proteins from bean. - J. biol. Chem. **270**: 10432-10438, 1995.

Garrett, J.L., Roseman, A.M., Saibil, H.R.: A small heat shock protein stably binds heat denatured model substrates and can maintain a substrate in a folding-component state. - EMBO J. **16**: 659-671, 1997.

Handschumacher, R.E., Harding, M.W., Rice, J., Drugge, R.J.: Cyclophilin: a specific cytosolic binding protein for cyclosporin A. - Science **226**: 544-546, 1984.

Huang, W., Fang, X.D., Zhao, W.M., Lin, Q.F.: [The methods of identifying differences in mRNA expression.] - Chin. J. Biotechnol. **18**: 521-524, 2002. [In Chinese.]

Hubank, M., Schatz, D.G.: Identifying differences in mRNA expression by representational difference analysis of cDNA. - Nucl. Acids Res. **22**: 5640-5648, 1994.

Leonardo, A.M.Z., Baudo, M.M., Palava, E.T., Heino, P.: Isolation and characterization of a cDNA corresponding to a stress-activated cyclophilin gene in *Solanum commersonii*. - J. exp. Bot. **49**: 1451-1452, 1998.

Li, M.S., Lee, S.Y.: Mangroves of China: a brief review. - Forest Ecol. Manage. **96**: 241-259, 1997.

Lin, P.: The physiological characteristics of salt tolerance for *Kandelia candel* seedlings. - In: Lin, P. (ed.): Mangroves Research papers (III). Pp. 150-154. Xiamen University Press, Xiamen 1999.

Lin, Q.F., Deng, Y.C., Huang, W., Chen, J.P., Li, G.Y.: [Introducing total DNA of *Rhizothora apicularta* to *Solanum melongena* to generate salt-tolerant progenies.] - Progress Biotechnol. **21**: 40-44, 2001. [In Chinese.]

Maurel, C.: Aquaporins and water permeability of plant membranes. - Annu Rev Plant Physiol Plant mol Biol. **48**: 399-429, 1997.

Mei, S., Wong, K.C., Lee, J.S.Y.: Reproductive biology and population genetic structure of *Kandelia candel* (*Rhizophoraceae*), a viviparous mangrove species. - Amer. J. Bot. **85**: 1631-1637, 1998.

Sobrado, M.A.: Hydraulic properties of a mangrove *Avicennia germinans* as affected by NaCl. - Biol. Plant. **44**: 435-438, 2001.

Stearns, T., Willingham, M.C., Botstein, D., Kahn, R.A.: ADP-ribosylation factor is functionally and physically associated with the Golgi complex. - Proc. nat. Acad. Sci. USA **87**: 1238-1242, 1990

Tyerman, S.D., Bohnert, H.J., Maurel, C., Steudle, E., Smith, J.A.C.: Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. - J. exp. Bot. **50**: 1055-1071, 1999.

Weig, A., Deswarte, C., Chrispeels, M.J.: The major intrinsic protein family of *Arabidopsis* has 23 membranes that form three distinct groups with functional aquaporins in each group. - Plant Physiol. **114**: 1347-1357, 1997.

Winicov, I.: New molecular approaches to improving salt tolerance in crop plants. - Ann. Bot. **82**: 703-710, 1998.