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Adaptation to salinity in mangroves: Implication on the evolution of salt-tolerance

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A plant's adaptation to its environment is one of the most important issues in evolutionary biology. Mangroves are trees that inhabit the intertidal zones with high salinity, while salt tolerance competence of different species varies. Even congeneric species usually occupy distinct positions of intertidal zones due to differential ability of salt tolerance. Some species have different ecotypes that adapt well to littoral and terrestrial environments, respectively. These characteristics of mangroves make them ideal ecological models to study adaptation of mangroves to salinity. Here, we briefly depict adaptive traits of salt tolerance in mangroves with respect to anatomy, physiology and biochemistry, and review the major advances recently made on both the genetic and genomic levels. Results from studies on individual genes or whole genomes of mangroves have confirmed conclusions drawn from studies on anatomy, physiology and biochemistry, and have further indicated that specific patterns of gene expression might contribute to adaptive evolution of mangroves under high salinity. By integrating all information from mangroves and performing comparisons among species of mangroves and non-mangroves, we could give a general picture of adaptation of mangroves to salinity, thus providing a new avenue for further studies on a molecular basis of adaptive evolution of mangroves.

mangroves, salt stress, adaptive evolution, gene, genome

Mangroves are trees inhabiting the intertidal zones of tropical and subtropical coasts[1]. They fall into two groups according to their habitats in nature: true mangroves and mangrove associates. True mangroves refer to species that specifically grow in intertidal zones, such as *Rhizophora apiculata*, *Kandelia candel*, *Ceriops tagal*, *Bruguiera gymnorrhiza*, *Aegiceras corniculatum*, *Sonneratia caseolaris*; while mangrove associates are capable of occurring in both littoral or terrestrial habitats, such as *Hibicus tilisaceus* and *Excoecaria agallocha*[2,3]. Mangrove species possess a common characteristic of tolerating high-salinity seawater, implying convergent adaptation of these species. Nevertheless, different mangrove species may adopt distinct strategies for adaptation to high salinity due to their differential ability of salt tolerance. How do mangroves adapt to high-salinity seawater and what are the underlying molecular mechanisms? What are the evolutionary forces that drive convergent adaptation in phylogenetically divergent mangrove species? These are critical questions in mangrove ecology and evolution. Here, we summarize some of the progress made in mangroves adaptation to salinity in terms of anatomical, physiological and molecular mechanisms, aiming to integrate relevant information in the physiological, genetic and genomic levels and to explore possible mechanisms of adaptive evolution of mangroves.

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1 Anatomic and physiological characteristics of mangroves and adaptation to salinity

It is important for mangroves to control cytosolic salt concentration when living in intertidal zones with high salinity[1]. Mangroves develop diverse mechanisms associated with anatomic or physiological characteristics to regulate salt absorption and exclusion, such as ultrafiltration, salt-secretion[1] and ion sequestration[4,5]. Some species can even accumulate saline ions as osmolytes[6] to balance transmembrane osmotic potentials. Diverse strategies of salt management indicate that mangroves are adaptive to high salinity adaptability in the anatomic and physiological levels.

Mangrove species usually have salt resistance-associated anatomic structures. One typical characteristic is thickening leaves that can reserve abundant water. For example, *Lumnitzera racemosa* can increase leaf thickness and water content when stressed with high salinity[7], by which absorbed salt was diluted and salt-induced damage was reduced to some extent. Waxed epidermis in the leaves is also a protective trait in some mangroves, which contributes to low transpiration efficiency of mangrove species relative to that of other plants without this trait. High salinity can remarkably slow down transpiration in some mangroves[8]. Salt gland in the leaves is another typical structure of some mangroves. Excessive saline ions can be secreted through this organ. All species in genus *Aegiceras*, *Avicennia*, *Acanthus* and *Aegialitis* have typical salt gland structures, and species in *Lumnitzera* and *Conocarpu* have analogous structure to salt glands. In addition, viviparity is also a characteristic that might be related to adaptation to high salinity. In some mangrove species (e.g. *K. candel*, *B. gymnorrhiza*, *B. sexangula*), salt concentration is lower in viviparous propagules than in other organs of mother trees[9], but it increases gradually during the process of propagule maturation[10,11]. It is suggested that when they are still attached to the mother trees, the progagules would be trained to tolerate high salinity by absorbing salinity from the mother trees[9]. All features mentioned above reveal higher competence of salt tolerance of mangroves in terms of morphology.

On a physiological level, recent studies show that mangroves can restrict cytosolic salt content not only by ultrafiltration[9,10,12−14], but also by other means such as salt accumulation and ion sequestration[4,5]. Salt controlling strategies in mangroves are similar to those in glycophytes, but probably, mangroves could exclude or sequester salt ions more efficiently.

Many mangrove species (e.g. *Kandelia obovata*, *Avicennia marinan*[6,15]) can accumulate inorganic ions and use them as osmolytes to maintain osmotic and water potential. This characteristic confers a survival advantage to these species in a saline environment[1]. After all, excessive ions in the cells would be harmful to the structure and activity of cytosolic proteins[16]. Thus, while sequestering excessive ions into vacuoles, mangroves could also accumulate organic osmolytes in cytoplasm to get osmotic equilibrium across the tonoplast. Organic osmolytes of mangroves mainly include hydroxyl compounds, free amino acids (especially, Proline), polysaccharide (e.g. starch), and so on. Relevant reviews have provided specific osmolytes for some mangrove species[17]. Here, we give a summary of osmolytes in mangroves (Table 1).

Table 1 Osmolytes in mangroves

Mangrove species	Osmolyte	Reference
True mangroves		
Kandelia candel	pinitol, mannitol	[18]
Rhizophora stylosa	pinitol, mannitol	[18]
Bruguiera gymnorrhiza	pinitol, mannitol	[18]
Bruguiera parviflora	proline	[19]
Aegiceras corniculatum	Starch or polysaccharide	[19]
	proline	[20]
	aspartic acid	[21]
Bruguiera sexangula	proline	[21]
Avicennia alba	proline	[21]
Xylocarpus granatum	proline	[21]
Acanthus ilicifolius	proline	[21]
	sterol, and so on.	[15]
	aspartic acid	[21]
Avicennia mariana	proline	[21]
	betaine	[18, 22]
Ceriops tagal	proline	[23]
	pinitol	[22]
Sonneratia alba	mannitol	[24]
Acrostichum aureum	pinitol	[25]
Mangrove associate		
Pongamia pinnata	proline	[21]
Hibiscus tiliaceus	proline	[21]
	betaine	[22]

While inhabiting the intertidal zones, mangroves suffer diverse stresses resulting from high salinity, hypoxia, ultraviolet radiation, nutrition deficiency and so on. These primary stresses may lead to secondary oxidation stress, resulting in accumulation of reactive oxidate species (ROS), peroxidation of membrane lipids and inac-

tivation of proteins. However, enzymes involved in anti-oxidation and their corresponding genes are also activated positively. For example, NaCl and H_2O_2 caused upregulation of *Cat1* (peroxidase) and *Fer1* (feritin) in *Avicennia marina*[26]. In *Bruguiera gymnorrhiza,* the activity of superoxide dismutase (SOD) increased with salinity and stress time[27], and that of catalase also increased quickly with time[12]. With an increase in H_2O_2 content in salt stressed *Bruguiera parviflora*, the content and activity of these antioxidative enzymes, ascorbate peroxidase (APX), superoxide dismutase (SOD), guaiacol peroxidase (GPX), glutathione reductase (GR) also increased, but catalase (CAT) was downregulated by salt stress[28]; Correspondingly, expression of genes coding for these enzymes changed[29]. *Aegiceras corniculatum* is a mangrove species with salt glands. Salt secretion was enhanced by a salt increase in this species and interestingly, activity of APX, GPX, CAT decreased[30]. It is clear that mangroves can activate diverse components of their antioxidative system to eliminate H_2O_2 and restrict the accumulation of ROS. Oxidative stress is a secondary cross-point of diverse primary stresses and this raises some interesting questions. For example, what does individual primary stress contribute to this secondary response most? How does the cellular signal transduction system work to harmonize such stress with primary stresses in mangroves?

2 Molecular characteristics of mangroves and adaptation to salinity

The high adaptatability to salinity of mangroves can be partially explained by morphological, physiological and biochemical studies. However, these were insufficient to clarify the salt adaptation mechanism and its evolution. Recently, some progresses have been achieved in understanding the mechanism of salt adaptation in mangroves on a molecular level. Tables 2 and 3 list some relevant studies in mangroves in the genic and genomic levels. These results indicated that mangroves adaptation to a high saline environment is indeed tightly linked to the regulation of gene expression. Here, we exemplify some cases to illustrate this point.

2.1 Salt secreting true mangroves

Avicennia marina is one of the most well-studied mangroves because of its characters of salt secretion and high salt tolerance. *A. marina* deals with salt stress through accumulating betaine serving as an osmolyte. Hibino et al.[18] first identified and cloned the *BADH* gene that is involved in betaine synthesis in *A. marina*. *BADH* was up-regulated under salt stress, and this tendency was consistent with the accumulation of betaine in *A. marina*. Two other genes, *AmT1* and *AmT2* (coding for Betaine/Proline transporter) were also isolated from *A. marina* later[31]. Mutant analysis, functional compensation test and protein or RNA detection in transgenic *E. coli* verified the functions of these genes. Meanwhile, transportation of betaine under NaCl or KCl treatment was enhanced in transgenic *E. coli* with *AmT1* and *AmT2* genes. Also, up-regulation of these two genes could be observed in *A. marina* leaves under high salinity[31]. All these observations indicated that the accumulation of betaine is related to the *AmT1* and *AmT2* genes in *A. marina*. On the genomic level, a large number of ESTs have been collected from adaptive seedlings of *A. marina* through techniques of differential display[32] or random sequencing of cDNA library clones[33]. Some genes in such EST collections, e.g. those coding for dehyrin and polypeptide hormone phytosulphokine, were continuously up-regulated after 48 hours of salt-stress[33]. Their transcript abundance returned to the normal level if salt stress was prolonged to 12—24 weeks, indicating adaptation of *A. marina* after long-term stress[33].

Aegiceras corniculatum is another species of high concern. Six hundreds of EST were obtained from the leaf SSH library of *A. corniculatum* under salt-stress[20]. *P5CS*, which was related to osmotic regulation, and two aquaprin genes, which participate in water transport[34], were up-regulated in *A. corniculatum* by salt stress[20]. Expression patterns of these 2 aquaprins also indicated that *A. corniculatum* could recover form long-term salt stress and adapt to saline environments[20]. There are several ongoing projects, including transfering *P5CS* and *CPI* (coding for cysteine proteinase inhibitor) genes of *A. corniculatum* into *Arabidopsis*, and microarray analysis of transcript profiling in *A. corniculatum*[35], could further help to depict mechanisms of adaptation and evolution in this species.

2.2 Non-secreting true mangroves

Bruguiera gymnorrhiza is a well-studied non-secreting true mangrove. Studies on its response to high salinty have been conducted recently at both the genic and genomic levels[36−42]. For example, *B. gymnorrhiza* OEE1 (oxygen evolving enhancer protein 1) protein was ini-

Table 2 Salt-related genes reported in mangroves

Mangrove species	Genes	Description	References
Aegiceras corniculatum	*P5CS*	delta 1-pyrroline-5-carboxylate synthetase, a key enzyme of proline synthesis pathway——Accumulation of transcript of this gene under high salinity tended to accompany recruitment of proline in *Aegiceras corniculatum*	[20]
	PIP1	PIP1 aquaporin——This gene was upregulated by salt stress	[20]
	PIP2	PIP2 aquaporin——This gene was upregulated by salt stress	[20]
	NHA	Na^+/H^+ antiporter——This gene was upregulated by salt stress	[20]
	CPI	cysteine proteinase inhibitor——Constitutive expression of this gene in transgenic Arabidopsis enhanced tolerance capacity in high saline medium	[35]
Avicennia marina	*BADH*	Betaine-2-aldehyde dehydrogenase——High salinity induced increase of transcript level and such an increase was accompanied by accumulation of betaine. Although activity of this enzyme decreased with an increase in salinity, the extent of decrease is less than its homologs in *E. coli* and spinage	[18]
	Sod1	Cu/Zn superoxide dismutase——High salinity did not lead to transcriptional change but osmotic stress decreased transcript level of this gene. Under oxidative stress, its transcription was transiently upregulated	[26]
	Cat1	catalase——It was upregulted by saline or oxidative stress but downregulated by osmotic stress	[26]
	Fer1	ferritin 1——It was transcriptionally Upregulated by saline or oxidative stress but didn't change under osmotic stress	[26]
	AmT1; *AmT2*; *AmT3*[partial]	Betaine/Proline transporter——Transgenic *E. coli* with such genes could accumulate betaine under salt stress; In *Avicennia marina*, salt stress induced transcription of such genes in root and leaf	[31]
Bruguiera gymnorrhiza	*OEE1*	OEE1 is one component of PSⅡ and high salinity induced accumulation of its transcript and protein	[36]
	DLDH	dihydrolipoamide dehydrogenase——Upregulated when treated with 500 mmol/L NaCl for 1 d	[39]
	LAS	lipoic acid synthase——Being upregulated when treated with 500 mmol/L NaCl for 1 d	[39]
	Unnamed gene	fructose-6-phosphate, 2-kinase/fructose-2, 6-bisphophatase——Transcription of this gene increased after 6 hours of salt stress. It was supposed to act in osmotic regulation process by controlling the content of Fru-2, 6-P_2	[39, 41]
	Cytosolic *Cu/Zn SOD*	cytosolic Cu/Zn superoxide dismutase——High salinity, mannitol and ABA induced accumulation of its transcripts in leaves; Transcript was induced by high salinity in young and mature leaves rather than in old leaves	[42]
	Cytosolic *CAT*（partial）	catalase——No significant change occurred in the expression of this gene during the treatment with NaCl, mannitol and ABA, but CEPA (2-chloroethylphosphonic acid) can increase its transcript level	[42]
Bruguiera sexangula	*CCTα*	α subunit of CCT complex——Transgenic *E. coli* with one domain of this subunit displayed enhanced tolerance to high salinity	[43]
	Mangrin	partially homologous to gene encoding Allene Oxide Cyclase (AOC)——It was upregulated by high salinity and its overexpression enhanced salt tolerance of transgenic yeast and tobacco cell	[43]

tially isolated and its corresponding gene was also obtained[36]. *OEE1* encoded the precursor of OEE1, which contained 322 amino acids. Active OEE1 functions together with *D1* in photosystem II (PSII). *OEE1* was up-regulated during 3 to 14 days after salt stress. However, the *D1* gene was not influenced simultaneously. Thus, it was assumed that the increase of *OEE1* transcripts would resume the OEE1 protein circulation and would be of benefit for stabilization of PSII system[36]. Currently there are also on-going genomic studies of *B. gymonorrhiza*. Miyama et al.[37] established the first *B. gymonorrhiza* EST library, which collected 14, 842 ESTs from leaves and roots after high salinity or hormone treatments. Clustering analysis generated 3 major categories of genes with specific expression patterns in response to 500 and/or 400 mmol/L NaCl treatment. The first category included a very small proportion of genes that up-regulated with all, 500 mmol/L NaCl, 400 mmol/L NaCl and various hormones, treatments in both leaves and roots. The second category was the largest one and these genes were up-regulated in leaves under various treatments but only in salt-stressed roots. The last category contained genes that were up-regulated by salt stress in both leaves and roots but down-regulated in leaves under hormone treatment. Such genes represented only a small proportion of ESTs collected from *B. gy-*

Table 3 Genome-scale studies in mangroves

Mangrove species	Description	Main point	Reference
Aegiceras corniculatum	constructing a leaf SSH library	This library contained 577 ESTs that are up-regulated by high salinity stress. Fourteen catagories were assigned for this EST collection and those categories of "protein synthesis", "defense", "transport", "ion homeostasis", "protein destination" and "signal transduction" were remarkable.	[20]
Avicennia marina	identifying differentially expressed genes in response to 50‰ seawater in leaves of 50d-old seedlings by differentially display technique.		[32]
	constructing a leaf cDNA library from 500 mM NaCl-treated seedlings.	Random sequencing generated 1602 ESTs which were grouped into 13 categories; Among these, 7% were homologous with stress-responsive genes.	[33]
Bruguiera gymnorrhiza	large-scale sequencing of ESTs collected from high salinity or hormone treated leaves and roots.	Assembly of 14, 842 high quality sequences generated 6943 unique genes and 62.5% of such EST collection matched known proteins in Blast searching. Totally 129 statistically-confident genes were grouped into 4 clusters depending on their EST frequency and each group has specific pattern of transcript profiling under high salinity.	[37]
	monitoring salt-responsive transcript profilings of 7029 unique genes in leaf and root tissues using microarray technique.	Clustering generated at least 6 categories of transcript accumulating under saline condition; Some genes displayed similar salt-responsive patterns to those in other plants, indicating shared mechanisms in *Bruguiera gymnorrhiza* and other glycophytes. Distinct expression patterns of other genes suggested existence of specific mechanisms in this species.	[38]
	identifying differentially expressed candidates through differentially display technique from leaves stressed with 500 mM NaCl for 0h, 6h, 3d, and 28d.	Totally 89 clones were identified as differentially expressed candidates; nine out of these candidates were verified by Northern Blot and classed into 3 groups depending on their salt-induced patterns of transcript accumulation	[39]
Ceriops tagal	constructing a leaf SSH library and a root cDNA library; monitoring time-course transcript profilings through microarray.	Totally 98 differentially expressed ESTs induced by 500 mM NaCl were identified and some of them were cooperatively regulated by salt to acting on tie-in processes. When compared with glycophytes, global transcription of this species is stable in saline environment, indicting homeostasis mechanism in salt-related adaptation.	[44]
Hibiscus tiliaceus	constructing a leaf cDNA library; monitoring time-course transcript profilings through microarray	Totally 1220 differentially expressed ESTs induced by high salinity were identified. Among these, 434 ESTs were assigned to function-known genes and some responded to high salinity in ecotype-specific manners. Genes of transcription factors responded to salt stress more rapidly, and changed much more in littoral ecotype than in terrestrial one.	[45]
Acanthus ebracteatus	constructing a leaf cDNA library from seawater-growing seedlings.	Random sequencing generated 521 readable sequences and 67% of them matched function-known genes by homolog searching; among which, 18% were predicted to function in stress response, 23.9% in metabolism, 7.3% in regulation of transcription and 2.7% in others.	[46]

monorrhiza libraries. Unique gene collections obtained from the assembly of those ESTs were later used in microarray experiments to monitor transcript profilings in leaves and roots of salt-stressed *B. gymonorrhiza*[38]. Totally, 228 genes displayed transcript levels five fold higher than in controls, while 60 genes were down-regulated to one fifth of control levels. Among these remarkably differentially expressed genes, only 32.5% up- regulated and 3.3% down-regulated genes were co-regulated in upper and lower leaves, as well as in roots[38]. The rest showed tissue-specific expression patterns[38]. Differing from EST analysis, differentially expressed genes in microarray analysis fell into 6 categories of gene expression patterns[38]. Interestingly, *Bg70*, appearing highly abundant in EST analysis, still showed salt-induced up-regulation in array analysis. However, gene encoding BURP-domain containing protein, which was also frequently expressed in EST analysis, appeared to be inhibited by high salinity in microarray analysis. This pattern was opposite to that of its homologous gene *RD22* in *Arabidopsis*[38].

Another non-secreting true mangrove species *Ceriops tagal* has also been of concern recently. More than 5000 EST clones have been obtained from its root cDNA library and leaf SSH library of *Ceriops tagal* [44]. By microarray analysis using chips containing this EST collec-

tion, time-course transcript abundance were monitored in roots[44] and leaves (Deng, et al, unpublished results) and differentially expressed genes were identified by comparing stressed *C. tagal* seedlings with controls. Additionally, comparison on global gene expression between *C. tagal* and *Arabidopsis* led to a suggestion that global expressional homeostasis might benefit *C. tagal* for adapting to high saline environments[44]. By comparing expression patterns of homologous genes between *C. tagal* and glycophytes, some genes were proposed as candidates possibly affected by natural selection (Liang et al., unpublished results). All these achievements could serve as starting points for further study on adaptive evolution of mangroves.

2.3 Mangrove associates

Mangrove associates represent transitional lifestyles between terrestrial plants and true mangroves and are ideal subject material to study the mechanism of adaptive evolution in plants. *Hibiscus tiliaceus* is such a species that has two ecotypes adapting to littoral and terrestrial habitats, respectively. Much more attention has been paid to it recently. Yang[45] constructed a leaf cDNA library of *H. tiliaceus* which generated approximately 8000 clones. Time-course transcript profiles were monitored through microarray techniques and 434 unique genes were identified as salt-responsive candidates[45]. It appeared that some genes responded to salt stress in an ecotype-specific manner. Notably, some candidates encoding transcription factors exhibited rapid and great up-regulation in the littoral ecotype but not in the terrestrial ecotype. Such positive correlation between transcription regulation and littoral ecotype implied upstream transcriptional regulation might contribute greatly to adaptive evolution in *H. tiliaceus* [45].

Large-scale analyses of EST and gene expression make it possible to perform comparisons between mangrove species or between mangroves and non-mangroves. For example, transcript profiles of *Ceriops tagal* (true mangrove), *Hibiscus tiliaceus* (mangrove associate) and *Arabidopsis* (glycophyte) showed remarkable differences among them. With stress for 24 hours, high salinity could induce a continual departure from the normal situation in wild-type *Arabidopsis*, but in *Hibicus tilisaceus*, expressional dispersion transiently changed with time and recovered quickly. Interestingly, dispersion of global transcription in salt tolerant *Ceriops tagal* was not evident (Liang et al. unpublished result). Even if transcriptional dispersion truly happened in *Ceriops tagal* it must have been changed very early and recovered immediately, because it could not be detected after stress for 2 h. Apparently, homeostasis of transcription might be responsible for adaptation to salinity in *Ceriops tagal* and this might be a common behavior of mangrove species to adapt to a saline environment. Additionally, some genes in mangroves exhibited distinct patterns of gene expression from their homologs in *Arabidopsis*, indicating that specific regulation of transcription was associated with salt tolerance of mangroves. It could be anticipated that function-characterization of these candidate genes would help to find out how natural selection contributes in adapting to high salinity in mangroves.

3 Prospects

Mangroves play important roles in coastal protection, buffering erosion by ocean waves, lessening the damages of typhoones and so on. Additionally, mangrove community represents a unique and significant ecosystem as a transition between two contrasting communities (marine and terrestrial). These features make it a good model system for studying adaptive evolution of salt tolerance in plants.

Much progress has been made in morphology, physiology and ecology of mangroves in the past two decades. Recently, benefiting from the development of genetic and genomic techniques, studies at these levels are also conducted on several species of mangroves. Nevertheless, there are still many difficulties in gene manipulation in mangroves, which restricts further studies at the molecular level. There are also many questions which should be answered in the future. For example, what adaptive strategies are specifically developed in mangroves and what is the essential molecular mechanism? What gene or gene combinations could be selected for adaptation to high salinity and contribute to evolution of salt tolerance in mangroves? Comparisons among different mangrove species (e.g. true mangroves vs. mangrove associates), or between mangroves and non-mangroves at the genomic level could also be informative.

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