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Changes of plasma membrane ATPase activity, membrane potential and transmembrane proton gradient in *Kandelia candel* and *Avicennia marina* seedlings with various salinities

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Abstract: The salt-secreting mangrove, *Avicennia marina*, and non-salt-secreting mangrove, *Kandelia candel* were cultivated in sand with various salinities(0‰, 10‰, 20‰, 30‰, 40‰) for 60 d. Plasma membrane vesicles of high-purity in leaves and roots of *A. marina* and *K. candel* seedlings were obtained by two-phase partitioning. The function of the plasma membranes, the activity of ATPase, membrane potential and transmembrane proton gradient, at various salinities were investigated. The results showed that within a certain range of salinity(*A. marina* and roots of *K. candel*: 0—30‰; leaves of *K. candel*: 0—20‰), the activity of ATPase increased with increasing salinity, while high salinity (above 30‰ or 20‰) inhibited ATPase activity. In comparison with *A. marina*, *K. candel* appeared to be more sensitive to salinity. The dynamics of membrane potential and transmembrane proton gradient in leaves and roots of *A. marina* and *K. candel* seedlings were similar to that of ATPase. When treated directly by NaCl all the indexes were inhibited markedly: there was a little increase within 0—10‰(*K. candel*) or 0—20‰(*A. marina*) followed by sharp declining. It indicated that the structure and function of plasma membrane was damaged severely.

Keywords: salinity; *A. marina*; *K. candel*; plasma membrane; H-ATPase; Ca-ATPase; membrane potential; transmembrane proton gradient

Introduction

For mangroves grow in subtropical and tropical coastal areas, high salinity is the most distinct characteristic of their growth environment. In this high salinity environment, mangroves have developed two kinds of salt-tolerating mechanisms: salt-secreting and non-salt-secreting (Lin, 1997). Some researchers have reported on the relationship between salinity and mangrove morphology (Das, 1993), growth (Zheng, 1993) and plasma membrane peroxidation (Zheng, 1997; 1998; Wang, 2000c). However, there are few reports on the relationship between the cell plasma membrane function of two kinds mangroves and salinity. Plasma membrane of plant cells, as the most important barrier between plant cells and its external environment, is closely related to plants resistance to stresses. Therefore, studies on the relationship between mangrove plasma membrane function and salinity may provide further information on the mechanisms for mangroves' salt tolerance.

There have been many studies on the relationship between plasma membrane ATPase of plant cells and salt stress. Comparative study on plasma membrane ATPase of salt-tolerant *Atriplex L.* and non salt-tolerant *Pisum L.* showed that, they had no difference in optimum pH and appetency to ATP, but the latter was much more sensitive to salinity than the former(Lerner, 1985). It is considered that the increase in ATPase activity of plasma membrane and vacuole membrane in plant root cells was one of the major factors for plant resistance to high salt stress, regulation of ion absorption and localization(Zhang, 1993). Wang *et al.* (Wang, 2000b) found that the accumulation of proline in *Ficus carica L.* cells under salt stress depended on the increase in ATPase activity of plasma membrane, and suggested that both the plasma membrane H^+-ATPase and vacuole membrane H^+-ATPase pumped H^+ out of cytoplasm jointly, thus increased pH, and then promoted the synthesis of proline. Investigation on the response of Ca^{2+}-transport system to salt stress showed that, salt stress affected Ca^{2+}-ATPase activity directly (Hao, 1993). Plasma membrane ATPase therefore plays a very important role during the physiological process of plant resistance to salt stress.

In the present study, we investigated comparatively the response of plasma membrane H^+-ATPase and Ca^{2+}-ATPase activities, membrane potential and transmembrane proton gradient of two kinds of mangroves (salt-secreting and non-salt-secreting) to various salinities.

1 Materials and methods

1.1 Long-term experiment: salinity treatment

Sand was collected from Jiulong River, washed thoroughly with tap water and then taken into porcelain pots (2.5 kg/pot) for four salt treatment: 0‰, 10‰, 20‰, 30‰ and 40‰, each treatment has three replicates. Mature seeds of *Avicennia marina* and *Kandelia candel* were collected from the firth of Jiulong River, Fugong Town, Longhai County, Fujian Province(24°29'N, 17°55'E). The seeds were sterilized with 0.1% sodium hypochlorite solution followed by thorough wash with deionized water, then planted in sand, 10 *A. marina* or 6 *K. candel* seeds for each pot. The four treatments for salinity were prepared by diluting seawater(salinity 20%) or by adding NaCl to seawater. The control (0‰) was irrigated with tap water. The seedlings were grown in greenhouse and the pots were rearranged

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randomly for several times. Sixty days after sowing the seedlings were harvested.

1.1.1 Preparation of high-purity plasma membrane

The plasma membrane of high-purity was prepared following the method of Sandelius and Morre (Sandelius, 1990) and Zheng *et al*. (Zheng, 2000). The harvested seedlings were divided into leaves and roots, rinsed thoroughly, and the fresh weights were determined. The fresh shoots or roots were then homogenized with two-fold of buffer containing Na_2EDTA 3 mmol/L, sucrose 0.25 mmol/L, PVP K-30 0.6%, PMSF 1 mmol/L, Tris-Mes 15 mmol/L (pH 7.8). The filtrated tissue extracts were centrifuged at 10000 × g for 15 min, the supernatants were further centrifuged at 50000 × g for 30 min. The residue was suspended by buffer containing 5 mmol/L phosphate buffer(pH 7.8), 0.1 mmol/L DTT, 1 mmol/L KCl. The suspending solution was mixed by ratio of 1∶3 (v/v) with solution containing dextran T-500 6.2%, PEG 4000 6.2%, sucrose 0.25 mol/L, KCl 3 mmol/L, K_3PO_4 5 mmol/L and was mixed by drastic inversion for 20—30 times followed by centrifuged at 1500 × g. The supernatant was collected carefully, diluted to 3—5 times by buffer containing Tris-Mes 5 mmol/L(pH 7.2), DTT 0.1 mmol/L, sucrose 0.25 mol/L, centrifuged at 100000 × g for 30 min. Collected residue was suspended by buffer containing Tris-Mes 5 mmol/L(pH 7.8), DTT 0.1 mmol/L, sucrose 0.25 mol/L. The whole procedure was carried out at 0—4℃.

1.1.2 Assays of enzyme activities

H^+-ATPase activity was assayed according to the method of Zhang(Zhang, 1997).

While Ca^{2+}-ATPase activity was measured according to Li(Li, 1995). The activities were expressed in $\mu mol\ L^{-1}$ pi. mg^{-1} pro. min^{-1}.

1.1.3 Measurement of membrane potential and transmembrane proton gradient

The membrane potential was measured using fluorescence probe Safrannine O according to Qiu and Chen (Qiu, 1997). The transmembrane proton gradient was measured using fluorescence probe Quinacrine according to Suzuiki *et al*. (Suzuiki, 1993).

1.2 Short-term experiment: direct NaCl treatment

A. marina and *K. candel* leaves were collected from the firth of Jiulong River, Fugong Town, Longhai County, Fujian Province(24°29′N, 117°55′E) and the high-purity plasma membrane was extracted as described above. NaCl solution of different concentrations(0 mmol/L, 175 mmol/L, 350 mmol/L, 525 mmol/L and 700 mmol/L, paralleled to 0‰, 10‰, 20‰, 30‰ and 40‰ for long-term experiment respectively) was added to the reaction solution directly on the plasma membrane.

2 Results

2.1 ATPase activities in roots and leaves of *A. marina* and *K. candel* seedlings under various salinities

Fig.1 shows the effects of salinities on ATPase activities in leaves and roots of *A. marina* and *K. candel* seedlings. Under salinities of 0‰—30‰, H^+-ATPase and Ca^{2+}-ATPase activities of both *A. marina* and *K. candel* were increased with increasing salinity, while they were inhibited at high salinity. At salinity of 30‰, H^+-ATPase activities of *K. candel* and *A. marina* were increased 169% and 135% compared with the control.

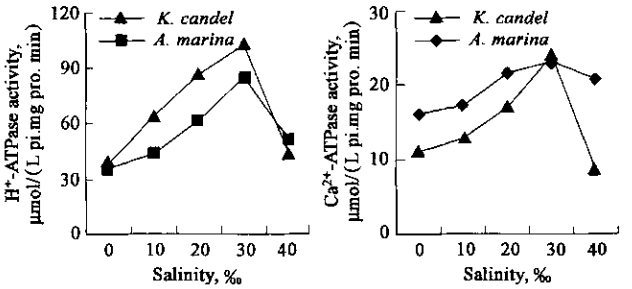

Fig.1 Effects of salinity on H^+-ATPase, Ca^{2+}-ATPase, activity in root plasma membrane of *A. marina* and *K. candel* seedlings

The ATPase activities in leaves of *A. marina* and *K. candel* seedlings were different from those in roots(Fig.2). For *K. candel*, at salinity of 0‰—20‰, the activity of ATPase was increased significantly, and decreased at high salinity(above 20‰). At the highest salinity of 40‰, both H^+-ATPase and Ca^{2+}-ATPase activities were reduced close to the level of the control. For *A. marina* however, the ATPase activities were still increased at salinity of 20‰—30‰, at high salinity(>30‰), they also declined but still higher than the control.

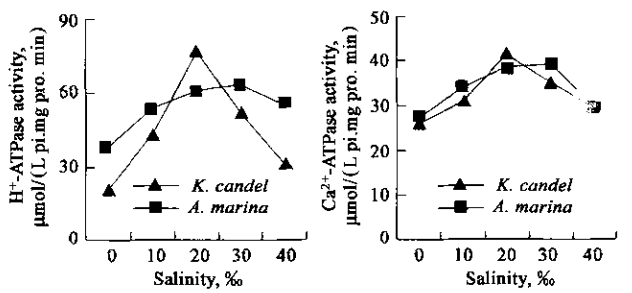

Fig.2 Effects of salinity on H^+-ATPase, Ca^{2+}-ATPase activity in leaf plasma membrane of *A. marina* and *K. candel* seedlings

2.2 ATPase activities in leaves of *A. marina* and *K. candel* seedlings under various NaCl treatments

When the membrane vesicles were treated with NaCl solution directly, the activities of ATPase in the membrane were inhibited significantly (Fig. 3). At low NaCl concentrations of 0—175 mmol/L(for *K. candel*,) or 0—350 mmol/L (for *A. marina*), ATPase activities were increased marginally, then were reduced markedly at high NaCl concentratons. At NaCl concentration of 700 mmol/L, the Ca^{2+}-ATPase activities were reduced 50.7% for *K. candel* and 42% for *A. marina*.

2.3 Membrane potential and transmembrane proton gradient in roots and leaves of *A. marina* and *K.*

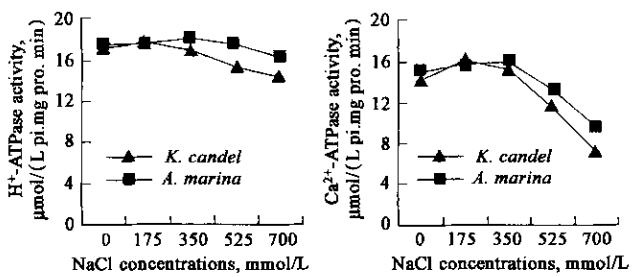

Fig.3 Effects of NaCl concentration on H^+-ATPase, Ca^{2+}-ATPase activity in leaf plasma membrane for *A. marina* and *K. candel* seedlings

***candel* seedlings under various salinities**

The change patterns of membrane potential and transmembrane proton gradient in roots and leaves of *A*. *marina* and *K*. *candel* seedlings under various salinities was similar to that of ATPase(Fig. 4). Under salinities of 0‰—30‰, the membrane potential and transmembrane proton gradient in roots of both *A*. *marina* and *K*. *candel* were enhanced with the increasing salinity (expressed in Q % · mg^{-1} pro·min^{-1}), they were also reduced at high salinities above 30‰. In leaves, the critical concentration was at 20‰ for *K*. *candel* and 30‰ for *A*. *marina*.

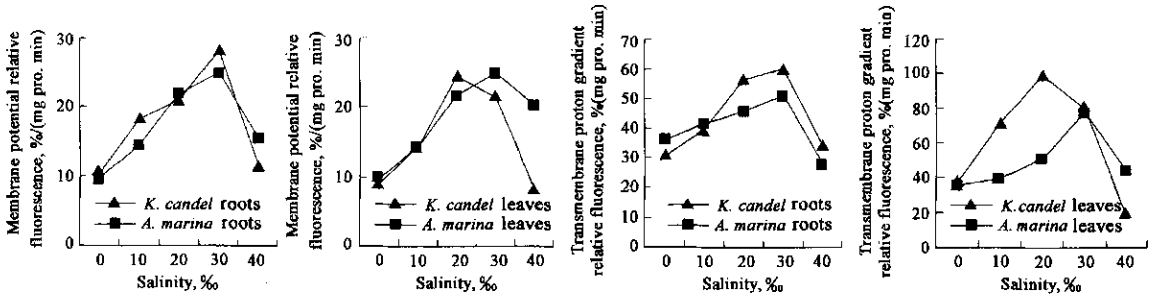

Fig. 4 Effects of salinity on membrane potential and transmembrane proton gradient in plasma membrane of roots and leaves for *A*. *marina* and *K*. *candel* seedlings

2.4 Membrane potential and transmembrane proton gradient in leaves of *A*. *marina* and *K*. *candel* seedlings under direct NaCl treatments

NaCl treatments also significantly affected membrane potential and transmembrane proton gradient in leaves of both *A*. *marina* and *K*. *candel* seedlings (Fig. 5). The membrane potential and transmembrane proton gradient were stimulated slightly at low NaCl concentrations(< 175 mmol/L for *K*. *candel* and < 350 mmol/L for *A*. *marina*) and were then significantly inhibited at high NaCl concentrations, especially for *K*. *candel* with inhibition by 36% and 59% at 700 mmol/L.

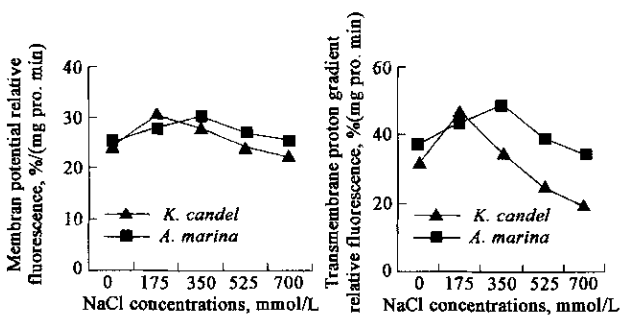

Fig. 5 Effects of NaCl concentration on membrane potential and transmembrane proton gradient in leaf plasma membrane for *A*. *marina* and *K*. *candel* seedlings

3 Discussion

The response of ATPase to salt stress was similar in both *A*. *marina* and *K*. *candel*. Under low salinities (0‰—20‰ for *K*. *candel* leaves and 0‰—30‰ for *A*. *marina* leaves and roots and *K*. *candel* roots), the plasma membrane ATPase activities were stimulated, but were inhibited at high salinities, and that the change of ATPase activities of *K*. *candel* appeared to be more remarkable with various salinities or NaCl concentrations compared with *A*. *marina*. Direct treatment of NaCl on membrane vesicles showed similar effects on ATPase activities, which was in agreement with the results of Wang *et al*. (Wang, 2000b). ATPase, as one of the most important parts of plasma membrane functions, plays an important role in the response to stress, especially H^+-ATPase. Many types of stress such as salt stress, low temperature, drought and light stress will affect the activity of H^+-ATPase. Investigations showed that the synthesis of such materials for osmotic regulation as proline and glycerolhas had close relation to plasma membrane ATPase activity (Chen, 1991; Wang, 2000a; 2000b). So far, it is not conclusive yet on how H^+-ATPase response to salt stress. Braun *et al*. (Braun, 1986) reported that, under salt stress, activity of plasma membrane H^+-ATPase in roots of *Atriplex L*. was increased by two folds, pH curve and kinetic characteristic were also changed, which suggested that salt stress changed the characteristic of H^+-ATPase. While Bruggmann *et al*. (Bruggmann, 1988; 1989) found that, salt stress did not change any characteristic of H^+-ATPase. Studies of Yamashita and Matsumoto (Yamashita, 1997) showed the plasma membrane H^+-ATPase activity of barley roots was reduced from 20% to 30% under 200 mmol/L salt stress for one day. The contents of total phospholipid and cholesterol in plasma membrane remained unchanged but the relative content of H^+-ATPase protein was reduced. So it was suggested the reduction of H^+-ATPase under salt stress was not because of changes in H^+-ATPase characteristic but of the reduction in H^+-ATPase protein. Our present experiment showed that, as halophyte, H^+-ATPase activities of both *A*. *marina* and *K*. *candel* increased under certain low range of salinity for long term and declined at high salinity, which was in agreement with our another experiment on nitrate reductase(NR; Zhao, 2001); while under short-term of direct NaCl treatment, H^+-ATPase activities were inhibited at lower NaCl concentrations, which was different from that under long-term salinity treatment. Accordingly we suggested both H^+-ATPase protein and some of H^+-ATPase characteristics such as structure and kinetics were affected. We considered that, maybe under long-term salt stress the H^+-ATPase protein change was dominant, appropriate salinity simulated synthesis of protein, while under short-term direct NaCl treatment, effect of ions toxicity dominated, which may result in change in enzyme structure and kinetics character, consequently the enzyme activity was changed.

Ca^{2+}-ATPase also plays an important role in alleviating salt stress(Pfeiffer, 1995). It was considered that Ca^{2+} take response for salt stress as one kind of messenger materials (Lauchi, 1990). Studies of Zhang *et al*. (Zhang, 1993) showed that Ca^{2+} could enhance root respiration and H^+-

ATPase activity and regulate absorption and transport of ions such as Na^+, K^+, Ca^{2+}. Hao and Yu (Hao, 1993) suggested one of the main reasons for salt stress resulting in the damage was the reduction in pumping Ca^{2+} ability of Ca^{2+} transport system. Accordingly, we suggested the enhancement of Ca^{2+}-ATPase activity in both *A. marina* and *K. candel* may be the result of feedback adaptability of its response to Ca^{2+} messenger regulation under salt stress for long time, thus it maintained certain Ca^{2+} level in cytoplast, and consequently guaranteed normal function of cells.

The results of the response of the membrane potential and transmembrane proton gradient in leaves and roots of *A. marina* and *K. candel* to various salinities were consistent with the data of ATPase activities. Under certain range of salt stress, both of the two indexes were stimulated and then decreased under salinity of above 20‰ (for leaves of *K. candel*) or 30‰ (for roots of *K. candel* and for leaves and roots of *A. marina*). Membrane potential and transmembrane proton gradient were the primary driving power of transmembrane transportation of all kinds of ions, amino acids and saccharide etc., their enhancement under salt stress may be also the result of salt resistance adaptability of mangroves. The establishment of membrane potential and transmembrane proton gradient were based on the function of ATPase, so the increase or decrease of ATPase activity inevitably resulted in the enhancement or reduction of membrane potential and transmembrane proton gradient. This experiment provided evidence for the hypothesis. Fluorescence probe Quinacrine takes on faint alkalescence, it can combine with protons pumped out of plasma membrane, which may lead to fluorescence abrupt extinction. The relative value of fluorescence abrupt extinction indicates the activity of proton transport expressed as transmembrane proton gradient. Enhancement of membrane potential and transmembrane proton gradient under certain salt stress promoted the trasmembrane transport and absorption of nutrients, which provide guarantee for plant normal life.

4 Conclusions

The plasma membrane ATPase activity, membrane potential and transmembrane proton gradient in leaves and roots of both salt-secreting *A. marina* and non-salt-secreting *K. candel* were affected by salinity. All the indexes of non-salt-secreting *K. candel* changed more notably than those of salt-secreting *A. marina*.

Under short-term NaCl treatment, the plasma membrane structure and function of both salt-secreting *A. marina* and non-salt-secreting *K. candel* were damaged. The present investigation indicated that both salt-secreting *A. marina* and non-salt-secreting have certain dependence on salinity. Their requirement for salt may be of both ecological and physiological significance. In comparison, non-salt-secreting *K. candel* was more sensitive and salt-secreting *A. marina* was more resistive to salinity.

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