Physiological adaptability of three mangrove species to salt stress

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Abstract: The impact of salinity on three arboreal mangrove plants, Sonneratia apetala (Sa), S. caseolaris (Sc) and Rhizophora stylosa (Rs), was studied. The three mangrove species were treated with different salinity levels over a three-month period. The response and adaptation of these three mangrove species to salinity were shown to be different. Net photosynthesis rate, stomata conductance and transpiration rate of leaves decreased and soluble sugar content in leaves increased, with salt concentration in all three mangrove species. The malondialdehyde (MDA) content in stems and leaves of Sa and Sc somewhat decreased when the salinity was lower than 10, but rapidly increased with increasing salt concentration. The MDA content in stems and leaves of Rs increased only when salinity was greater than 40. No changes were observed in the MDA content of roots in the three mangrove species. The adaptabilities of Sa and Sc to salt tolerance were limited. The more salt tolerant the mangrove Rs, the more likely the free oxygen radicals were eliminated through the increase in activity of superoxide dismutase (SOD). Results of this experiment identified salinity levels best suited for the growth and metabolism of the species, which provides information necessary for maintaining mangrove forestation along the South China coast.

Key Words: Sonneratia apetala; S. caseolaris; Rhizophora stylosa; salt stress; net photosynthesis rate; stomata conductance; transpiration rate; soluble sugar; membrane peroxidation; SOD

Mangroves, a kind of xylophyte, form unique communities in tropical and subtropical regions and tidal lowlands. Ecologically, they play an essential role in protecting adjacent land from wave and storm erosion[1,2] and providing habitat and food for fish, prawns, crabs, shellfish and birds. They can also filter surface water from inland, and reduce pollutants in offshore waters[3].

Mangrove plants generally grow in a special environment with certain salinity and consequently have their special adaptation system, different from land plants and freshwater plants. It is generally thought that mangrove plants grow in certain salinity conditions and the adaptation of different species to salinity is different[4,5]. Above or below certain salinity level, their growth will be inhibited and even death can occur. Under salt stress, that is, beyond appropriate salinity, either low or high, the flexibilities and physiological reactions of different mangrove species are dissimilar. The major physiological characteristics, such as photosynthesis, protein synthesis and energy metabolism, are also more or less affected[6].

Sonneratia apetala (Sa), S. caseolaris (Sc) and Rhizophora stylosa (Rs) are three dominant mangrove species on the coastland of South China. Of them, Sa and Sc were introduced from Bengal, and have become the most important species in afforestation along coastal areas. In this article, research on the physiological reaction of the three mangrove species with various treatments of salinity has been carried out and the relationship between different physiological reactions is explored, which helps to reveal the effect of salinity changes on the three species and provides a reference index for the choice of salt tolerant species.

1 Materials and methods

1.1 Materials

Seedlings of Sa, Sc and Rs were collected from the mangrove nature reserve (19°51’N, 110°24’E), Dongzhai Gulf in Hainan Island, China, at the end of January 2006, and all the
seedlings were one-year-old with similar size. Seventy-two seedlings of each mangrove species were planted in plastic pots with 60 kg clay and every pot contained nine seedlings. They were irrigated daily for three months with six liters of brine, whose salinity varied from 0 to 50, that is, 0, 5, 10, 15, 20, 30, 40 and 50. Water was emptied every night. Every several days water was replenished to keep the salinity stable. They were placed in natural light. Every 30 days, brine was replaced by water with the same salinity. The apparatus (Orion 4 star) was used to analyze the water quality. The total time period was 90 days.

1.2 Methods

Salt scale: use water to dissolve sea salt, and use WYY35T salinometer to rectify salinity.

Net photosynthesis rate, stomata conductance and transpiration rate were measured with the help of Li-6200 portable photosynthesis apparatus (made in USA). The mature, stable leaves were chosen as research objects and measured, with temperature controlled between \((20 \pm 2) \, ^\circ\text{C}\), light quantum flux between 600–800 \(\mu\text{mol}/(m^2\cdot s)\), relative humidity between 60\% \pm 5\%, and CO\(_2\) density \(3.6 \times 10^4\).

The total soluble sugar content was measured using the anthrone colorimetry\[7\]. The extracts were gained by referring to Lin’s method\[8\].

Protein content was measured according to Bradford’s method\[9\]. Coomassie brilliant blue G250 dye and colorimetry were used at the wavelength of 595 nm. Bovine serum albumin (BSA) was used as standard protein. Protein content was expressed by mg/g fresh weight.

The upper lucid extracts served as the materials for measuring the activity of superoxide dismutase (SOD) and the content of malondial dehyde (MDA). The above operations were carried out in a temperature between 0–4 \(^\circ\text{C}\). Heath’s methods were used as reference in the measurement of membrane peroxidation function, and MDA was used as index of membrane peroxidation function\[10\]. MDA content was calculated according to the MDA mole extinction coefficient \(\Delta\varepsilon\) (532–600 nm) = 155 \(\mu\text{mol}^{-1}\cdot\text{cm}^{-1}\) and was expressed by mmol/mg protein. The method which Beauchamp and Fridovich\[11\] established was used to measure the activities of SOD. By suppressing reducing agent NBT, 50% was taken as an enzyme activity unit, and the enzyme activity was expressed by unit/mg protein.

The measurements of all indices were repeated at least thrice.

2 Results and analysis

2.1 Effects of salinity on leaf net photosynthesis rate, stomata conductance and transpiration rate of \(\text{Sa}, \text{Sc}\) and \(\text{Rs}\)

The indices of leaf net photosynthesis rate, stomata conductance and transpiration rate of the three mangrove species were measured at the same time (9 a.m., April 27, 2006). It was found that photosynthesis was inhibited by high salinity. Net photosynthesis rate and stomata conductance of \(\text{Sa}\) and \(\text{Sc}\) decreased as salinity in the culture medium rose from 0 to 40 (Figs. 1 and 2). When salinity was above 15, the decreasing tendency was obvious. Net photosynthesis rate of \(\text{Rs}\) increased appreciably in the beginning (< 20) and decreased subsequently (> 20), but the changes tended to be minor. Stomata conductance of \(\text{Rs}\) increased to 10, the highest level in salinity, but the overall changes were not obvious. In high salinity, in all three mangrove species, the metabolic flow with the outside slowed down, which was shown by the fact that net photosynthesis rate and stomata conductance decreased and these plants completed metabolism by accumulating internal CO\(_2\). According to the changing tendency, in low salinity (< 15), the net photosynthesis rate and stomata conductance of \(\text{Sa}\) and \(\text{Sc}\) were relatively stable, which showed the adaptation of the two species to low salinity. Net photosynthesis rate and stomata conductance of \(\text{Rs}\) rose to the highest level in salinity, 20 and 15, or even higher salinity, they did not change visibly, which was possibly related to the salt rejection in the roots of \(\text{Rs}\), that is, salt did not get to the leaves and consequently had less effect on \(\text{Rs}\) than on \(\text{Sa}\) and \(\text{Sc}\).

Transpiration rate of \(\text{Sa}\) and \(\text{Sc}\) decreased with an increase in salinity. Transpiration rate of \(\text{Sc}\) decreased sharply in low salinity, but transpiration rate of \(\text{Rs}\) kept steady (Fig. 3). This tendency was similar to that of the effects of salinity on sto-
mata conductance (Fig. 2), which indicated that the decrease of stomata conductance affected transpiration rate to some extent, and enhanced the mangrove’s function of conserving water in high salinity. Rs stability of transpiration rate proved that metabolism was kept at a stable level.

### 2.2 Effects of salinity on total soluble sugar content in leaves of Sa, Sc and Rs

Through cultivating three mangrove species with various salt concentrations for three months, it was discovered that total soluble sugar content in leaves of Sa, Sc and Rs all tended to rise with increase in salinity. Total soluble sugar content in leaves of Sa and Sc increased by a larger margin in high salinity (> 30) than in low salinity (< 30). For Rs, total soluble sugar content increased steadily (Fig. 4).

This result indicated that water scarcity stress enhanced as salinity increased (from 0 to 40). To control the balance of ions in the vacuole, the cells accumulated a type of low, compatible molecular compound, which took the place of water as a solvent in biochemical reactions and protected cell structure and water circulation [12]. As was shown in the experiment, with the salinity above 30, soluble sugar content in leaves of Sa and Sc increased, thus improving the permeability and maintaining a balance of water metabolism [13]. However, soluble sugar content in leaves of Rs was always at a low level, which could have resulted from the salt rejection of Rs, that is, it was difficult for the salt to get to the leaves, and the total soluble sugar content did not change a lot. Or it could be explained as the accumulation of other compatible solutes, such as praline [14,15], glycine [16,17] and polyol [18,19].

### 2.3 Effects of salinity on membrane peroxidation and activities of SOD of roots, stems and leaves of Sa, Sc and Rs

It was discovered that membrane peroxidation was affected remarkably by salt stress in leaves and stems of Sa, Sc and Rs. MDA content in leaves and stems of Sa and Sc decreased slightly in low salinity (< 10) and then increased rapidly (Figs. 5 and 7), which was reverse to the activities of SOD (Figs. 6 and 8).
and 8). It was thus evident that, although membrane peroxidation was affected remarkably in leaves and stems of Sa, Sc, no more SOD was generated, which might be related to the types of the plants or possibly to the fact that Sa and Sc made other peroxisome or catalase protect membrane peroxidation from being destroyed with the help of active oxygen free radicals.

Dissimilar to the changing tendency of salinity effects on MDA content in leaves and stems of Sa and Sc, MDA content of Rs decreased in low salinity (< 40) and increased only when salinity was higher than 30 (Fig. 9). It was because high salinity affected the metabolism system balance of active oxygen, increased the content of active oxygen, and consequently the membrane structure was destroyed by the superoxide anion free radicals, and then the peroxide was produced, for instance. For Rs, under high salinity stress, SOD was more active (Fig. 10) and the changing tendency was reverse to that of the salinity effect on the MDA content (pertinence coefficient $R^2 = 0.893$). It showed that Rs mainly depended on SOD to remove the active oxygen free radicals and SOD activity in leaves and stems directly affected the MDA content. When salinity was above 40, the membrane protection system was destroyed, the SOD activity decreased and the MDA content increased.

Changes in MDA content and activities of SOD in roots of three mangrove species showed that they were insensitive to salinity. The reason is that the mangrove plant roots are submerged in seawater perennially and the roots don’t have enough oxygen to absorb, and therefore mangrove plants have developed a root metabolism system, which has adapted to the special environment. This kind of root metabolism system has possessed a special physiological adaptation mechanism, which can be explained by the relatively small changes in the membrane peroxidation of roots in high salinity.

To summarize, when salinity is higher than 40, the mangrove species Rs can have some adaptations or endurance to salt stress, the function of membrane protection system is improved in the stems and leaves or almost kept at a high level in the roots, and therefore, the damage that various free radicals cause to membranes is kept at a small degree, which protects the normal cell function and ensures the seedlings with a normal growth. When the salinity becomes much higher, the function of the membrane protection system decreases, various free radicals gradually cause great damage to the membrane, and membrane peroxidation becomes obvious. All this causes a disturbance in the normal metabolism, a weakness in cell function, and a decrease in adaptation and endurance to salinity stress. It can be concluded that under salt stress, in seedlings of Rs, free radicals do cause damage to membrane, namely, membrane peroxidation and protection resulted from SOD or other systems influence each other. In comparison with Rs, Sa and Sc have a little bad salt tolerance, and SOD does not have strong protection ability for membrane.

### 3 Discussion

The physiological characteristics of the three mangrove species were greatly affected with various salt treatments. Its overall tendency was that changes in Sa and Sc were more obvious than in Rs, which proved that Rs adaptability to high salinity was much stronger. Sa and Sc could survive in high salinity, but high salinity deeply influenced their growth and metabolism.

In the field of phytophysiology, many studies have been carried out on the effects of salinity on physiology of man-
grove species, and also on effects of salinity on stomata conductance and transpiration. Kotmir and Nazaenko found that high salinity inhibited photosynthesis by closing the stomata and partially suppressing RUBISCO’s activity. High temperature, intense light, drought and high salinity proved to be able to stimulate genes related to heat shock proteins in plants and in mangrove plants, too. Several studies showed that salt stress could weaken photosynthesis of mangrove plants. There were also studies that with salt concentration, photosynthesis is not weakened and even in low salinity, photosynthesis is enhanced. The difference is mainly related to the species, and the salt stress exerts different effects on different species.

The reasons for the decrease of photosynthesis rate are mainly as follows: first, dehydration in cell membrane decreases permeability to CO₂, second, it is because of salt toxicity; third, closure of the stoma causes the decrease of CO₂; fourth, salt concentration quickens senescence; fifth, changes in cell structure cause changes in enzyme activity. Decrease in the photosynthesis rate also results from the decrease of stomata conductance, which causes the lack of CO₂ necessary for carboxyl reaction. Closure of the stoma reduces water evaporation, affects photosynthesis of the chloroplast and impacts energy transformation system, thereby changing the activity of the chloroplast. To what extent the closure of the stoma affects the photosynthesis rate depends on the partial pressure of CO₂ in the lamina. However a study reported that under salt stress, photosynthesis decreases because stomata opening is not being inhibited. Because of enhancing the transfer resistance to CO₂ and decreasing the efficiency for RUBISCO, stoma opening is not affected. According to the feedback of other salt-induced reactions, inhibiting the metabolism processes of some carbons can also decrease photosynthesis.

Another mechanism that mangrove plants overcame under high permeable pressure, in an environment with high salinity, was accumulating a compatible solute. Popp and other researchers studied 23 types of mangrove plants and discovered that pinitol and mannitol were the most common compatible solutes. They also discovered proline in Xylocarpus plants, methyl quaternary ammonium compounds in two kinds of Avicennia plants, Acanthus ilicifolius and Heritiera littoralis, and also in Hibiscus tiliaceus. As a compatible solute, glycine was discovered in Avicennia marina.

As is shown by experiment results in this article, when salinity is higher than 30, total soluble sugar content in leaves of Sa and Sc increases, thus improving the permeability and maintaining a balance of water metabolism. However, total soluble sugar content in leaves of Rs is always at a low level and this could result from the salt rejection of Rs, so salt does not have a lot of effect on total soluble sugar content. Otherwise, it could be explained as the accumulation of other compatible solutes.

Active oxygen includes superoxide, hydrogen peroxide and hydroxyl, which can be induced by extreme environment stress, such as extreme temperature, herbicide, drought and nutrient stress. Some higher plants resist active oxygen by improving the activity of the antioxidizing enzyme. SOD catalyzes the transformation of superoxide to hydrogen peroxide and then hydrogen peroxide is decomposed by the hydrogen peroxide enzyme and peroxide enzyme. However, there are very few reports about effects of salt stress on the active oxygen mechanism. Superoxide toxicity holds that on the one hand, active oxygen free radicals can cause oxidation of membrane and damage the membrane structure and normal cell physiology, but on the other hand, a membrane protection system also exists and slows the damage. This membrane protection system is in fact an anti-oxidation system, which generates different antioxidizing enzymes including the important SOD. SOD can effectively eliminate the active oxygen free radicals and prevent the membrane from damaging by oxidation.

The research of Zhao indicated that MDA content of the plant increased with the increase of salt concentration, whereas the activity of SOD and ATP enzyme reduced with the increase of salt concentration, which proved the existence of a damage mechanism of free radicals to terraneous plants. This research showed that the activities of SOD were inversely correlated to the MDA content in organs of salt tolerant mangrove plants Sa, Sc and Rs (Figs. 5–10). The overall tendency in Sa and Sc was that membrane peroxidation increased with the increase of salt concentration and the activities of SOD decreased at the same time. In Rs, when salinity was above 40, the damage of membrane peroxidation became obvious.

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