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# Relationship of leaf ultrastructure of mangrove *Kandelia candel* (L.) Druce to salt tolerance

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**ABSTRACT**: The leaf ultrastructure of mangrove *Kandelia candel* (L.) Druce planted in pots under different salinity conditions was compared under a transmission electron microscope (TEM). The results showed that the plasmalemma in plants grown in salinity conditions of 0‰ treatment (control) and 25‰ treatment was tightly combined, while in plants with salinity of 50‰ treatment, the plasmalemma crimpled remarkably and plasmolysis occurred. The nucleus and its two-layer membranes were obvious in control plants. In the case of 25‰ treatment, the membrane breakdown was observed, nucleoplasm dispersed in cytoplasm, and the electron density of cells was lower than that in control plants. In plants treated with 50‰ salinity the nucleus collapsed and no structure of the nucleus could be observed. As far as chloroplasts in control plants were concerned, they were oblong with a typical arrangement of grana and stroma thylakoids and one or two grains of starch. However, the chloroplasts in plants treated with 25‰ salinity were swelling and usually contained more grains of starch and few plastoglobuli. Most chloroplasts had a reduced number of grana, particularly of thylakoids in grana as compared with control plants. The chloroplasts of plants treated with 50‰ salinity had a considerably reduced system of grana and stroma thylakoids, and sometimes they were even deformed morphologically. They were mixed-up and contained more grains of starch and plastoglobuli. The indistinct structure of mitochondrial cristae was observed only in plants treated with 50‰ salinity. These showed that mitochondria are cell organs less sensitive to hypersaline conditions than chloroplasts and nucleus, and it was deduced that respiration was more conservative to an environment change than photosynthesis.

**Keywords**: *Kandelia candel* (L.) Druce; salt stress; mesophyll cell; ultrastructure

The mechanisms of salt tolerance in plant cells have been extensively described from ecological and physiological perspectives and from cellular and molecular biological aspects (Flowers et al. 1977; Niu et al. 1995; Hasegawa et al. 2000). Plants with some salt tolerance respond with their morphological structure. For example, mangrove species of *Aegiceras, Acanthus, Avicennia* and *Aegialitis* are salt-excretors, with the corresponding morphological and anatomical structure to adapt themselves to saline habitats. Salt glands existing in their leaves function to secrete salt (Lin 1999). It was suggested that a salt gland was composed of five parts: collecting cell, basal cell, secreting cell, collecting chamber and salt gland sheath (Ye et al. 1988).

Mangroves are the thermophilic coastal woody plant communities in tropical and subtropical coastal regions and tidal lowland (Lin 1999). Their physiological features and effects related to salinity are important fields of international mangrove research. Obvious effects of salinity on mangrove growth, water metabolism, photosynthesis, respiration were investigated (Lin 1999). *Kandelia candel* (L.) Druce is a dominant mangrove species on the

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eastern coast of southern Fujian. Some knowledge of the ultrastructure of mesophyll cells from leaves of two mangrove species *Kandelia candel* and *Aegiceras corniculatum* in relation to effects of salinity below 20‰ was obtained (Ye, Lin 1995). However, the average seawater salinity in the main distribution areas of mangroves in China is 33‰~34‰ in Hainan, 32.5‰ in eastern Guangdong, 30.0‰ in western Guangdong and 27.6‰ in annual average surface water in Xiamen Harbor, Fujian Province (Lin 1988). Leaves are organs for photosynthesis and of great importance to their survival. External morphology and internal structure correspond with their physiological function. To adapt the change of environment and survival conditions, leaf structure will also change. So this paper aims to study the relationship of leaf ultrastructure of the mangrove species *Kandelia candel* to salinity above 20‰.

## MATERIALS AND METHODS

### Plant materials and culture conditions

Mature propagules of *Kandelia candel* (L.) Druce were collected during April from the mangrove forest at the Jiulongjiang River Estuary (24°24´N, 117°55´E), Fujian, China. The propagules of similar length (20~25cm) were planted in 0.15cm diameter pots filled with sand at a density of three propagules per pot. Temperatures ranged from 25°C to 32°C during the day and 20°C to 25°C by night. They were irrigated daily with normal tap water under natural day and night for 2 months. Then the plants were grown at respective salinities of 0.25‰ and 50‰ with each treatment of 4 pots for 20 days. Leaves for measurements were young, fully expanded, and selected from the second nodes (from top to bottom).

### Transmission electron microscopy

Samples of different treatments from the second nodes were hand cut and fixed in a 5% glutaraldehyde solution buffered in 0.1M phosphate buffer solution (PBS), pH 7.0, for 48h at room temperature. After washing, the samples were postfixed for 24h in 1% osmium tetroxide in the same buffer, dehydrated in grade ethanol and embedded in epon 812. Ultrasections were cut on an LKBV ultramicrotome and then they were stained with uranyle acetate and lead citrate. Finally, ultra-thin sections of leaf tissue were examined and photographed with JEM-1010 transmission electron microscope (TEM).

## RESULTS

### Cell and nucleus

After different salinity treatments there were apparent changes in the cell ultrastructure. The cell wall and plasmalemma in plants grown in salinity conditions of 0‰ treatment (control) and 25‰ treatment were tightly combined (A, B). However, in plants with salinity of 50‰ treatment, the plasmalemma crimpled remarkably and plasmolysis occurred (C).

As far as the nucleus was concerned, it was sensitive to saline conditions. The nucleus and its two-layer membranes were obvious in control plants. Both the nucleus and the nucleolus were big (A). The nucleus and nucleolus of plant treated with 25‰ salinity were also big, however, the membrane of the nucleus began to break down, nucleoplasm dispersed in cytoplasm, and the electron density of cells was lower than that in control plants (B). In plants treated with 50‰ salinity the nucleus collapsed and no structure of the nucleus could be observed (C).

### Chloroplasts

Chloroplasts of control plants did not exhibit any abnormalities on the ultrastructure level of their organization. They were oblong, with a typical arrangement of grana and stroma thylakoids and one or two grains of starch (D). The chloroplasts in plants treated with 25‰ salinity were also oblong. These chloroplasts were swelling and usually contained more grains of starch and few plastoglobuli. The electron density of the stroma of the majority of chloroplasts was lower than in control plants. Such chloroplasts were numerous in mesophyll cells located in the zone of vascular bundles. Most chloroplasts had a reduced number of grana, particularly of thylakoids in grana as compared with control plants (E). The chloroplasts of plants treated with 50‰ salinity had a considerably reduced system of grana and stroma thylakoids, and sometimes they were even deformed morphologically. Few grana thylakoids were closely combined and compact, and sometimes they looked as if they were merging. They were mixed-up and contained more grains of starch and plastoglobuli (F).

### Mitochondria

Mitochondria were rich in plants grown in 0‰ and 25‰ salinity. They are rounded. Inner and outer membranes of mitochondria were distinguishable.

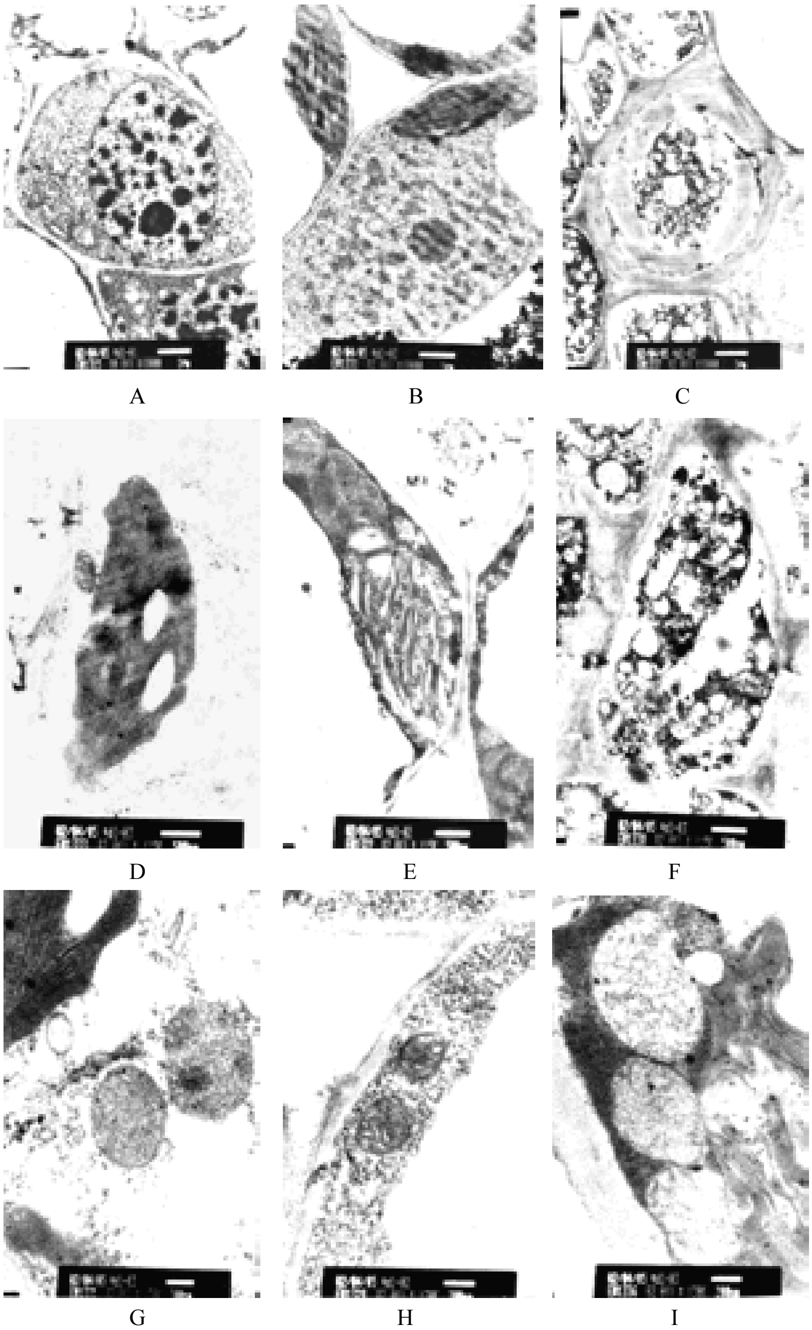


Fig. 1. Effects of salinity tolerance on the leaf ultrastructure of mangrove *Kandelia candel* (L.) Druce

Explanation:

A – Showing the intact cell with distinguishable nucleolus and two-layer membrane of nucleus in plants treated with 0‰ salinity (×8,000)

B – Showing the cell with nucleus and two-layer membrane out of focus in plants treated with 25‰ salinity (×8,000)

C – Showing the cell with mixed-up nucleus in plants treated with 50‰ salinity (×8,000)

D – Showing chloroplasts with distinguishable grana and stroma thylakoids in control plants (×15,000)

E – Showing chloroplasts with swollen thylakoids and increased number of starch grains in plants treated with 25‰ salinity (×15,000)

F – Showing chloroplasts with mixed-up thylakoids and accumulation of plastoglobuli in plants treated with 50‰ salinity (×15,000)

G – Showing mitochondria with distinguishable inner and outer membranes, and cristae in control plants (×30,000)

H – Showing mitochondria with distinguishable inner and outer membranes, and cristae in plants treated with 25‰ salinity (×30,000)

I – Showing mitochondria with distinguishable inner and outer membranes in plants treated with 50‰ salinity and cristae in mitochondria were still clear (×30,000)

Mitochondrial cristae were also clear (G, H). Mitochondria in plants with 50‰ salinity were also round-shaped. Their inner and outer membranes were still distinguishable, but mitochondrial cristae were out of focus (I).

## DISCUSSION

Reduction and swelling of thylakoids in plants as a reaction to excess salt were observed in our experiment. The same phenomena on plants as a response to excess Ni were obtained in cabbage (Molas 1997) and in pine and birch (Kukkola, Huttunen 1998; Kukkola et al. 2000).

Energy supply to cells affected by the unfavourable environment resulted in injury to the photosynthetic membrane, of which starch was the most sensitive product in chloroplast response (Jia 1990). This was confirmed by the fact that the number of starch grains increased with the exposure level in our experiment. It may be suggested that starch could not only mitigate the energy deficiency and keep the normal life working under salt stress but also increase the osmotic pressure to absorb water and sustain it. It should be stressed that the influence of salinity caused the accumulation of starch, which may indicate the inhibition of carbohydrate transport from leaves to roots because it was accompanied by the presence of quite numerous plastoglobuli (Molas 2002).

It was proposed that plastoglobuli were composed of the products of membrane breakdown (Barton 1966). The increased accumulation of plastoglobuli in chloroplasts and thylakoids mixed-up of plants treated with high 50‰ salinity observed in our experiments represented one feature of tolerance of *Kandelia candel* seedlings to the salt environment. It is known that the content of plastoglobuli increased in stress conditions, including stress caused by hypersaline conditions (Jia 1990) and nickel (Molas 1997; Kukkola, Huttunen 1998). Plastoglobuli can increase the concentration of cytoplasm, reduce the osmotic potential, and make cells absorb water and inorganic nutrients. So the accumulation of plastoglobuli is clearly one of most important strategies employed by mangrove *Kandelia candel* (L.) Druce against salt stress.

The structure of nucleolus and two-layer membrane of nucleus in control plants was distinguishable. With the increase of salinity in treatments, they began to disaggregate and nucleoplasm dispersed in cytoplasm. Our results also showed that no structure of the nucleus could be found in plants grown in 50‰ salinity conditions. So it may be deduced that the nucleus as one-cell organ may be sensitive to the hypersaline environment.

It is interesting to find that in hypersaline conditions, inner and outer membranes of mitochondria in plants were still obvious. This showed that mitochondria were less sensitive organs to high salinity as compared with chloroplasts and nucleus. It is well known that mitochondria play mainly the role in the activity of plant respiration. Therefore it may be deduced that respiration is more conservative to the environment change than photosynthesis.


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## Závislost mezi ultrastrukturou listu kořenovníku obecného *Kandelia candel* (L.) Druce a tolerancí k soli

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**ABSTRAKT**: Pod transmisním elektronovým mikroskopem (TEM) jsme porovnávali ultrastrukturu listů *Kandelia candel* (L.) Druce, který jsme vysázeli do nádob s rozdílnými podmínkami salinity. Výsledky ukázaly, že plazmalemy u rostlin pěstovaných v podmínkách salinity 0 ‰ (kontrola) a 25 ‰ byly pevně spojené, zatímco u rostlin ve variantě 50‰ salinity se nápadně kroutily a docházelo k plazmolýze. Jádro a jeho dvouvrstevné membrány byly u kontrolních rostlin zřetelné. V případě 25‰ salinity jsme zjistili porušení membrány, nukleoplazma se rozptýlila v cytoplazmě a hustota elektronů buňky byla nižší než u kontrolních rostlin. U rostlin pěstovaných při salinitě 50 ‰ docházelo k rozpadu jádra a nebylo možné zjistit strukturu jádra. Co se týče chloroplastů u kontrolních rostlin, byly podlouhlého tvaru s typickým uspořádáním grán a thylakoidů stromy s jedním nebo dvěma škrobovými zrny. Chloroplasty u rostlin ve variantě 25‰ bobtnaly a obvykle obsahovaly více škrobových zrn a několik plastoglobulů. Ve srovnání s kontrolními rostlinami většina chloroplastů měla snížený počet grán, zejména thylakoidů v gránech. Chloroplasty rostlin pěstovaných v podmínkách 50‰ salinity měly značně redukovaný systém grán a thylakoidů a v některých případech byly dokonce morfologicky deformované. Byly promísené a obsahovaly několik škrobových zrn a plastoglobulů. Nezřetelnou strukturu mitochondrií cristae jsme zjistili pouze u rostlin ve variantě 50‰ salinity. Ukázalo to, že mitochondrie jsou buněčný orgán méně citlivý na podmínky nadměrné salinity než chloroplasty a jádro a bylo možné odvodit, že respirace je konzervativnější vůči změně prostředí než fotosyntéza.

**Klíčová slova**: *Kandelia candel* (L.) Druce; solný stres; buňka mezofylu; ultrastruktura

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