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Anatomical responses in *Kandelia candel* (L.) Druce seedlings growing in the presence of different concentrations of NaCl

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Abstract. We studied the histology of leaves and roots of *Kandelia candel* seedlings growing in various salinities (0–550 mM NaCl). The structural changes found were related mainly to growth status. The plants grew best in the presence of 50 and 100 mM NaCl, with greater leaf area and thickness, higher ratios of palisade to leaf thickness, and higher stele to root cross-section-area proportions. Lignification and/or suberization of the cell wall appeared farther from the root apex. Structural modifications associated with adaptation to a saline environment were not found in this study—physiological regulation may be more important.

Keywords: Anatomical changes; *Kandelia candel*; Mangrove; NaCl; Rhizophoraceae; Salinity.

Introduction

Plants that tolerate high salinity environments are termed halophytes. They always evolve structural modifications and physiological adaptations (Flowers et al., 1986). Some of the structural changes that occur in those halophytes that lack specialized salt-excretion organs such as salt glands or salt bladders include increased succulence, changed number and size of stomata, thickened cuticle, inhibited differentiation, and changed diameter and number of xylem vessels. There may also be extensive development of tyloses and earlier occurrence of Casparian strips and lignification (Flowers et al., 1986; Poljakoff-Mayber, 1975). Depending on the species, these changes may be either adaptations to salinity stress or signs of damage and disturbance of the normal life processes (Poljakoff-Mayber, 1975).

The mangrove *Kandelia candel* (L.) Druce grows in the intertidal zone along estuarian river banks, where the tidal salinity ranges between 10 and 36 parts per thousand (ppt) (Hwang, 1983). The optimal salinity for the growth of *K. candel* seedlings, however, is between 0 and 15 ppt (ca. 257 mM NaCl) (Hwang and Chen, 1995). The basic anatomical structures of this species have been studied by Chiang (1984). Structural changes were noticed when seedlings collected from saline environment in the field were grown in freshwater in a greenhouse for more than one year. These plants showed decreased thickness of leaves and cuticle layers, reduced tannin content in the tannic and palisade cell layers, increased intercellular spaces, and increased ratio of spongy cell layers to leaf (Chiang, 1984). The anatomical structure of *K. candel* changes in response to different salinity conditions, but whether these changes are related to the development of salt tolerance was not clear. The present study investigates further the relationship between anatomical change and salt tolerance in *K. candel.*

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Materials and Methods

Mature propagules of *Kandelia candel* (L.) Druce, collected from mature trees growing along the Tamshui River, Taipei, Taiwan, (121° 26' E, 25° 9' N), were cultivated on sand in pots, partially submerged in nutrient solutions. The culture solution was a modified Hoagland's solution, with NaCl added at the beginning to form 0, 50, 100, 250, 400 and 550 mM NaCl solutions. Details of the culture method were described by Hwang and Chen (1995). We used mature leaves and roots of four-month-old plants for our study, except for the autofluorescence study, which used root tissues of one-month-old plants.

Light Microscopy

Leaf area was measured with a leaf-area meter (model Li-3000A, LI-COR Inc., Lincoln, Nebraska, USA). Leaf discs (7 mm diameter) were cut with a paper punch along the mid-rib at the central part of mature leaves. Segments (0.5 cm long) of mature root were cut 2 cm behind the hypocotyl, and segments (0.5 cm long) of root tip were cut 0.5 cm behind the apex. The segments were fixed for 8 h in FAA (37% formalin, acetic acid, 50% alcohol = 5:5:90). The materials were then dehydrated through a TBA (tertiary butyl alcohol) series and embedded in paraplast (Oxford Labware, Maryland, USA). Cross sections (10 μm) were cut with a rotary microtome (Leitz, West Germany) and stained with 1% safranin O and 0.5% fast-green (Johansen, 1940).

We used autofluorescence to assay areas such as cell walls of the exodermis, endodermis, and protoxylem for the presence of lignin and/or suberin (Peterson et al., 1981). Twelve roots were randomly selected from three plants (four from each). Root length was measured, and unstained fresh sections (100 μm) were cut in series from the root apex with a vibratome (Lancer Vibratome 1000, Brunswick Co., Missouri, USA) and observed with a light microscope (Model BHS, Olympus Optical Co., Tokyo, Japan) equipped with an epifluorescence optics (exciting wavelengths 400–440 nm).

Scanning Electron Microscopy (SEM)

Stomata of leaves were observed by SEM. Leaf discs were fixed by vacuum infiltration with 5% glutaraldehyde in 0.1 M phosphate-buffered solution (pH 7.2), then post-fixed in 1% (v/v) OsO_4 in 0.1 M phosphate-buffered solution. The fixed materials were then dehydrated by a 2,2-DMP and acetone fast-dehydration method (Lin et al., 1977). Dehydrated materials were critical-point dried with CO_2, mounted, and coated with a gold ion coater (Eiko Engineering IB-2, Ibaraki, Japan). Electron-microscopy was carried out using a Zeiss DSM-950 scanning electron microscope operated at an accelerating voltage of 20 kV.

Thickness of leaf and of palisade and spongy parenchyma tissues, and diameter of root, cortex, and stele were measured with an image processor. Stomata number per leaf was estimated as the product of stomatal density and leaf area. Mean values of each parameter for all salinity treatments were compared using the SAS general linear model procedure (PROC GLM) one-way analysis of variance and Tukey's Studentized Range test ($P \leq 0.05$; SAS, 1989).

Results

Leaf Anatomy

Stomata were found only on the abaxial side of the leaves. The stomatal density was significantly higher on plants growing in the absence of NaCl (Table 1), but because the leaf area of plants grown in the absence of NaCl was significantly smaller than that of those grown in low and medium salinity solutions, no differences in the estimated stomata number per leaf were found among plants grown in the presence of less than 250 mM NaCl (Table 1). Plants growing in 400 mM NaCl and above, however, had significantly lower leaf area and stomata number per leaf (Table 1). Apparently, the development of stomata and growth of leaf on plants growing in greater than 400 mM NaCl was inhibited.

The leaves of plants grown in low salinity were thicker than those of plants grown in high salinities (Table 2). The palisade-mesophyll ratio was highest for plants grown in 100 and 250 mM NaCl (Table 2, Figure 1). The spongy mesophyll portion, however, did not vary with salinity (Table 2). The increased leaf thickness seen in low salinity treatments seems to be a result of the increased thickness of the palisade mesophyll portion.

Root Anatomy

The mature-root cross section area did not show any trend associated with the salinity, although that of plants grown in the absence of NaCl was the largest (Table 3). The ratio of stele cross-section area to root section was higher in plants grown in 50–250 mM NaCl than in those

Table 1. Stomatal density, leaf area, and estimated number of stoma per mature leaf (n=4, ± s.d.) from *K. candel* grown in different concentrations of NaCl.

Salinity (mM NaCl)	Stomatal density (no./cm^2)	Leaf area (cm^2)	Stomata/leaf (no./leaf)
0	19905 ± 641^a	13.51 ± 0.24^c	268877 ± 3885^a
50	15231 ± 1304^b	16.13 ± 0.51^b	245304 ± 14100^a
100	14911 ± 265^b	18.35 ± 0.79^a	273512 ± 6956^a
250	15194 ± 1492^b	18.49 ± 0.70^a	280810 ± 27035^a
400	14133 ± 1277^b	13.33 ± 0.83^c	187642 ± 5690^b
550	16721 ± 600^b	10.88 ± 0.88^d	181788 ± 14325^b

Different superscripts indicate that differences between salinity treatments are significant according to the Tukey's Studentized Range test ($P \leq 0.05$).

Table 2. Leaf thickness and ratio of spongy- and palisadal-parenchyma to leaf thickness (n=8, ± s.d.) in mature leaves of *K. candel* grown in different concentrations of NaCl.

Salinity (mM NaCl)	Leaf thickness (μm)	Sponge/Leaf (%)	Palisade/Leaf (%)
0	390 ± 40^b	60.7 ± 5.2	21.1 ± 2.5
50	420 ± 40^a	57.6 ± 3.5	22.5 ± 2.8
100	390 ± 30^b	56.3 ± 3.2	26.2 ± 2.3
250	360 ± 20^c	51.5 ± 3.5	27.1 ± 2.3
400	390 ± 40^b	56.2 ± 4.0	24.5 ± 3.2
550	350 ± 20^c	56.0 ± 4.0	22.9 ± 3.8

Different superscripts indicate that differences between salinity treatments are significant according to the Tukey's Studentized Range test ($P \leq 0.05$).

Table 3. Mature root cross section area and the ratio of cortex and stele area to root cross section (n=6, ± s.d.) in *K. candel* grown in different concentrations of NaCl.

Salinity (mM NaCl)	Root area (mm^2)	Cortex/root (%)	Stele/Root (%)
0	3.9 ± 0.5^a	67.5 ± 5.5	10.9 ± 2.8
50	2.5 ± 0.6^c	52.0 ± 1.7	14.6 ± 2.8
100	3.1 ± 0.4^b	58.3 ± 2.8	14.4 ± 3.1
250	3.3 ± 0.6^b	66.4 ± 3.6	10.7 ± 2.9
400	2.5 ± 0.2^c	61.5 ± 3.3	9.3 ± 1.2
550	2.9 ± 0.2^{bc}	62.0 ± 2.8	6.4 ± 0.8

Different superscripts indicate that differences between salinity treatments are significant according to the Tukey's Studentized Range test ($P \leq 0.05$).

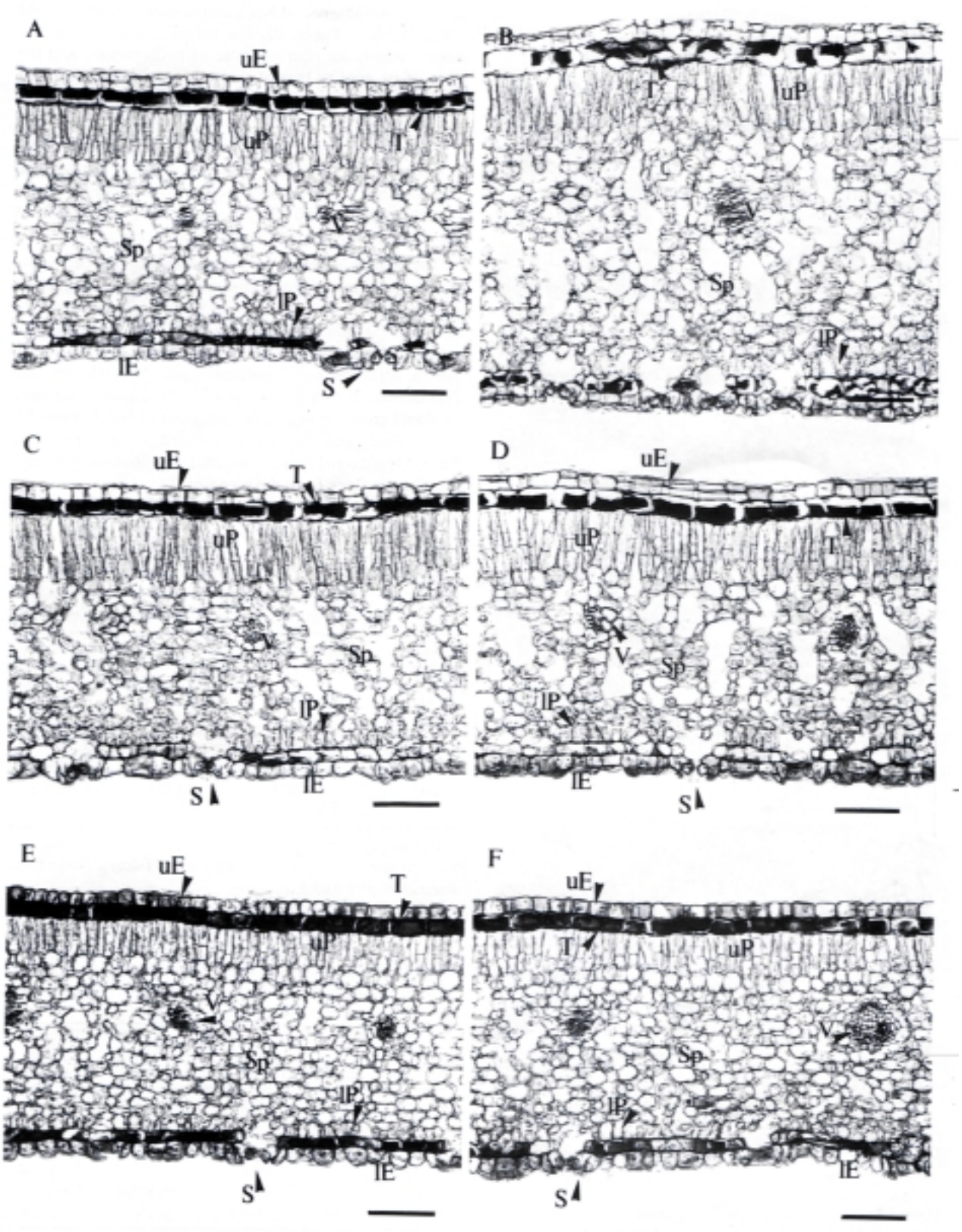

Figure 1. LM-micrographs showing cross sections of mature leaves from 4-month-old *K. candel* plants. Growth conditions were (mM NaCl): A, 0; B, 50; C, 100; D, 250; E, 400; and F, 550. Scale bar = 100 μm. **lE**, lower epidermis; **uE**, upper epidermis; **lP**, lower palisade parenchyma; **uP**, upper palisade parenchyma; **S**, stomata; **Sp**, spongy parenchyma; **T**, tannin layer; **V**, vascular bundle.

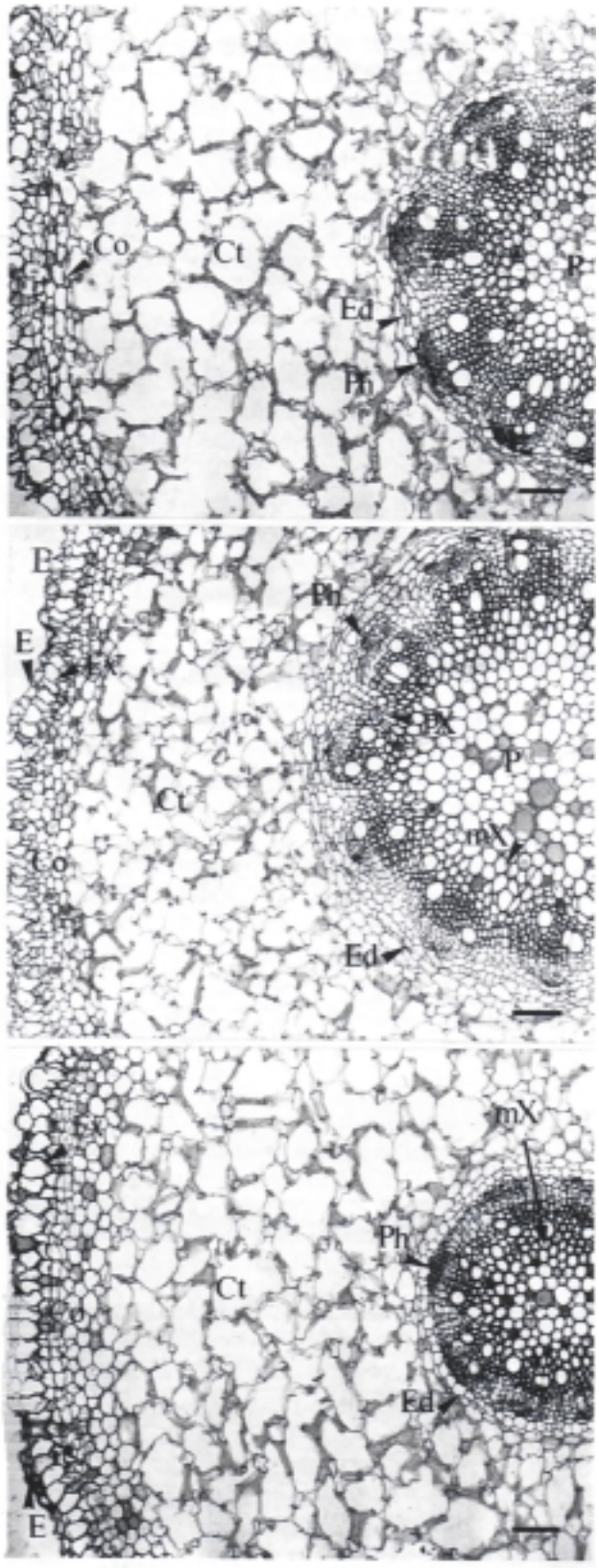

Figure 2. LM-micrographs showing cross sections of mature roots from 4-month-old *K. candel* plants. Growth conditions were (mM NaCl): A, 0; B, 100; C, 550. Scale bar = 100 μm. **Co**, cork layer; **Ct**, cortex; **E**, epidermis; **Ed**, endodermis; **Ex**, exodermis; **mX**, metaxylem; **P**, pith; **Ph**, phloem; **pX**, protoxylem.

grown in the absence of NaCl and in greater than 400 mM NaCl (Table 3, Figure 2). The cortex contained large lacunae, which are characteristic of hydrophytes, and the ratio of its area to that of the root cross section did not show any trend associated with the salinity (Table 3, Figure 2).

The cross-section area of root apical region (0.5 cm behind the tip) was larger in plants grown in greater than 250 mM NaCl. The root cross section area and the ratio of stele cross-section area to root section was largest in plants grown in 550 mM NaCl (Table 4, Figure 3).

The lignification and/or suberization of the cell walls of the exodermis, endodermis, and protoxylem within the root apical area was observed by UV autofluorescence. In all plants, autofluorescence was first observed in the protoxylem (Figure 4B) about 2 mm from the apex (Table 5), but the appearance of autofluorescence in exodermis and endodermis (Figure 4) was significantly closer to the apex in plants grown in high concentrations of NaCl (Table 5). High salinity, however, caused reduced root growth (Table 5), which could have affected the distance of the autofluorescence from the root tip.

Table 4. Apical root (0.5 cm behind the tip) cross section area and the ratio of cortex and stele area to root cross section (n=6, ± s.d.) in *K. candel* grown in different concentrations of NaCl.

Salinity (mM NaCl)	Root area (mm^2)	Cortex/root (%)	Stele/Root (%)
0	0.9 ± 0.4^{c}	60.6 ± 10.3	6.5 ± 1.4
50	0.7 ± 0.1^{c}	60.7 ± 7.2	5.8 ± 1.1
100	1.1 ± 0.6^{c}	60.4 ± 8.0	5.2 ± 1.7
250	2.3 ± 1.0^{b}	61.7 ± 6.9	5.3 ± 1.2
400	2.2 ± 0.9^{b}	58.4 ± 7.8	6.1 ± 1.6
550	3.0 ± 0.9^{a}	61.2 ± 4.3	8.8 ± 2.4

Different superscripts indicate that differences between salinity treatments are significant according to the Tukey's Studentized Range test ($P \leq 0.05$).

Table 5. The length of root and distance (mm, n=12, ± s.d.) to the root tip when the first autofluorescence appeared in root tissues of one-month-old *K. candel*.

Salinity	Root tissues			
(mM NaCl)	Length	Exodermis	Endodermis	Protoxylem
0	49 ± 4^{b}	2.6 ± 0.5^{ab}	4.8 ± 0.5^{ab}	2.1 ± 0.3
50	55 ± 5^{a}	2.7 ± 0.5^{a}	5.3 ± 0.6^{a}	2.2 ± 0.4
100	45 ± 4^{b}	2.3 ± 0.3^{ab}	4.5 ± 0.5^{ab}	1.9 ± 0.2
250	45 ± 5^{b}	2.4 ± 0.6^{ab}	4.4 ± 0.3^{b}	2.1 ± 0.4
400	36 ± 5^{c}	2.0 ± 0.3^{b}	3.6 ± 0.4^{c}	1.8 ± 0.3
550	31 ± 5^{c}	2.0 ± 0.5^{b}	3.6 ± 0.4^{c}	1.9 ± 0.4

Different superscripts indicate that differences between salinity treatments are significant according to the Tukey's Studentized Range test ($P \leq 0.05$).

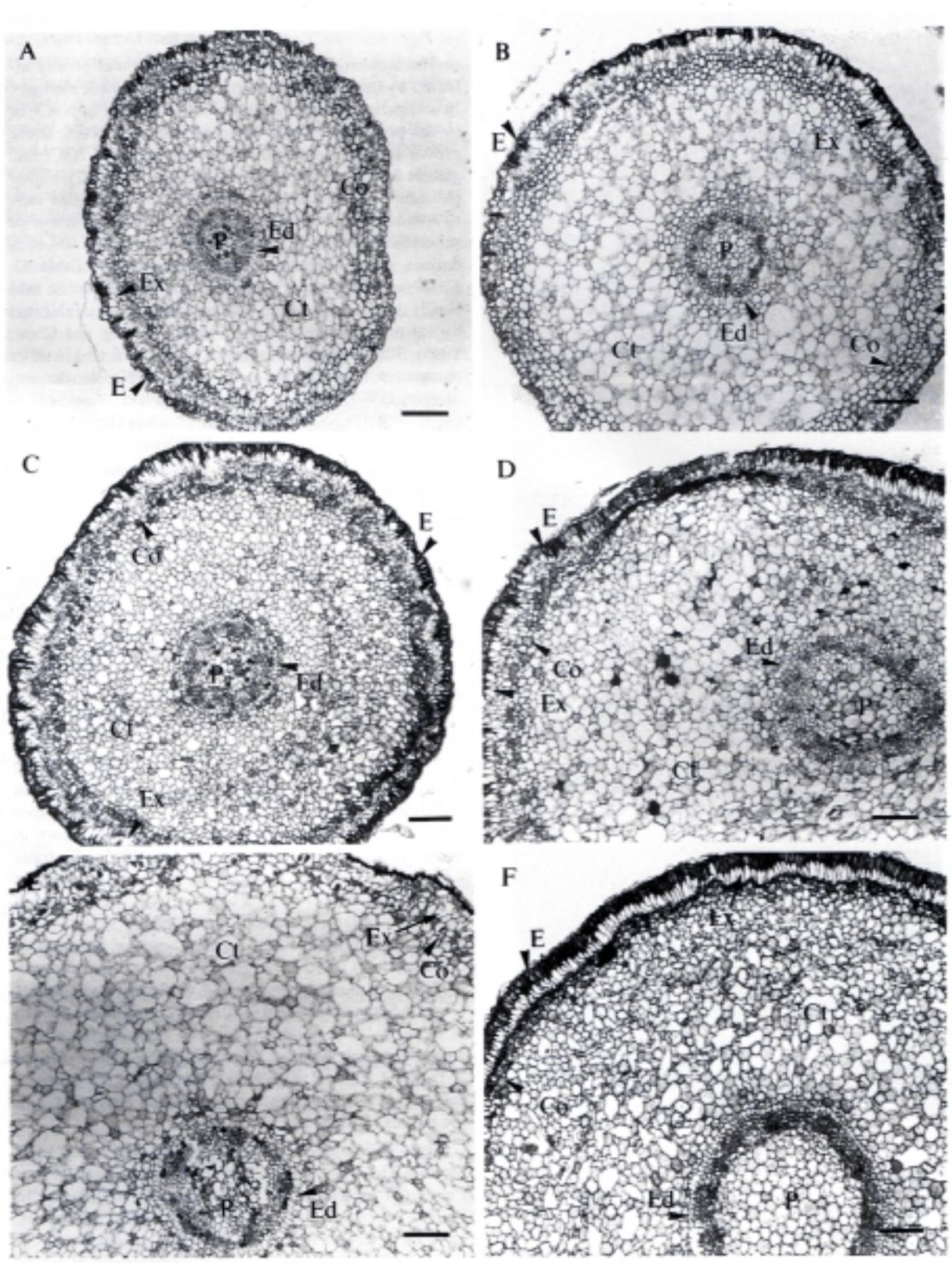

Figure 3. LM-micrographs showing cross sections of root tips from 4-month-old *K. candel* plants. Growth conditions were (mM NaCl): A, 0; B, 50; C, 100; D, 250; E, 400; and F, 550. Scale bar = 100 μm. Abbreviations are the same as for Figure 2.

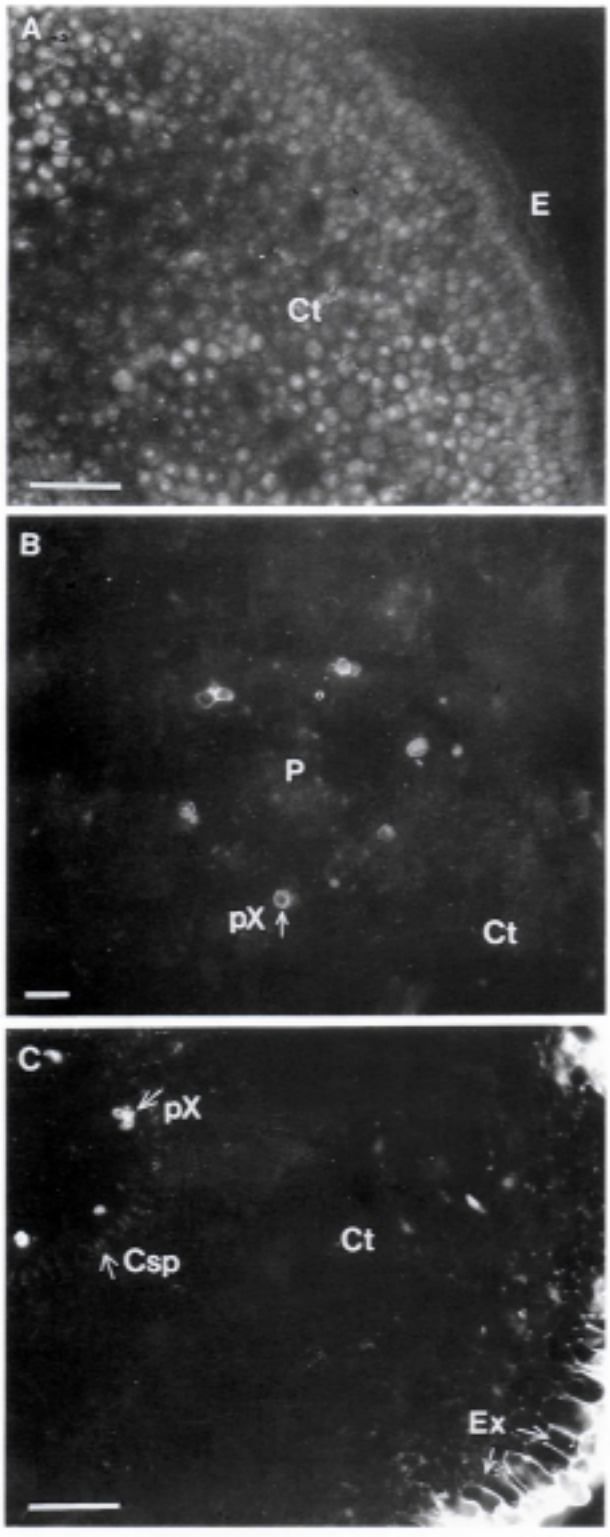

Figure 4. LM-micrographs showing autofluorescence in cross sections of root tips from 1-month-old *K. candel* plants. Growth conditions and distance to the apex (mM NaCl, mm) were: A, no autofluorescence (250, 1.2); B, protoxylem showing autofluorescence (50, 3.8); C, protoxylem, endodermis and exodermis showing autofluorescence (50, 3.2). Scale bar = 100 μm. **Csp** = Casparian strip; other abbreviations are the same as for Figure 2.

Discussion

The anatomical structure of *K. candel* was greatly affected by the NaCl concentration of the growth medium. In comparison to plants grown in the absence of NaCl, or in the presence of high concentrations of NaCl, those grown in low and medium concentrations of NaCl had greater leaf area (Table 1) and thickness (Table 2), higher palisade to leaf-thickness ratios (Table 2), and higher stele to root-section area ratios (Table 3). Lignification and/or suberization of the cell walls in the endodermis and exodermis appeared farther from the root tips (Table 5). *Kandelia candel* grows better in low salinity (about 86 mM NaCl) than in zero NaCl, and growth is greatly inhibited by salinities above 430 mM NaCl (Hwang and Chen, 1995). Similar growth responses were also found in other mangroves (Clough, 1984; Downton, 1982; Werner and Stelzer, 1990), although the beneficial role of sodium in the growth of C_3 halophytes is still unclear (Brownell and Crossland, 1972; Murata et al., 1992).

The aspects of *K. candel*'s leaf anatomy most obviously influenced by salinity were leaf area (Table 1) and leaf thickness (Table 2). High salinity caused lower stomatal number per leaf and reduced leaf thickness. These results, however, are not consistent with the findings of studies of other dicotyledonous halophytes, in which high salinity always caused increased leaf thickness and decreased stomatal density (Flowers et al., 1986). Popp et al. (1993) found a positive correlation between succulency and salinity in mangroves grown in the field, but not for those grown under glasshouse conditions. Whether the differences in leaf-response in *K. candel* are due to the differences in growth conditions needs further investigation.

The root tissues of other halophytes showed different growth responses to saline environments. *Suaeda maritima* grown in the presence of 340 mM NaCl had greater root stele diameter and cortical thickness than those grown in the absence of NaCl (Hajibagheri et al., 1985). *Prosopis tamarugo* showed smaller root diameter, lower cortex layer number, and smaller vascular-system size when grown in NaCl concentrations up to 400 mM (Serrato Valenti et al., 1991). In the present study, the larger ratio of stele to root cross section (Table 3, Figure 2) and greater root growth (Table 5) in plants grown in low and moderate salinities might indicate a beneficial influence of NaCl on the growth of *K. candel.* The trend was reversed when *K. candel* was cultured in high salinities (400 and 550 mM NaCl) (Table 2, Figure 1, Table 3, Figure 2). This could be the result of salt stress caused by the accumulation of Na^+ and Cl^- in the tissues (Hwang and Chen, 1995).

Early development of the Casparian strips, and their location close to the root apex was found in salt-grown *Puccinelli peisonis* (Stelzer and Lauchli, 1977) and in *Suaeda maritima* (Hajibagheri et al., 1985). Moreover, early lignification of secondary tissues, such as metaxylem, was believed to be a general characteristic of plants growing in habitats with a low water potential (Saadeddin and Doddema, 1986; Serrato Valenti et al., 1992). The in-

creased dimensions in the root apex region (Table 4, Figure 3) and the reduced distance of the Casparian strip on the endodermis to the root tip (Table 5, Figure 4C) found in *K. candel* grown in high salinities were consistent with those in *S. maritima* (Hajibagheri et al., 1985). *Kandelia candel* grown in high salinities, however, developed shorter roots than those grown in low salinities (Table 5), which is the reverse of findings with *S. maritima* (Hajibagheri et al., 1985). We suggest that the increased dimensions in the root apical region of *K. candel* is caused by an inhibition of cell division in the apical meristem.

Plants grown in high salinity environments face both ionic and water stresses. *Kandelia candel* does not possess salt glands, bladders, or other salt-secreting structures in its leaves or stem (Chiang, 1984)—absorbed salt accumulates in tissues and causes metabolic stress. In the absence of excretory organs, *K. candel* had to evolve other mechanisms of adaptation to survive in high ionic- and low osmotic-potential environments. We found no special structures in the roots and leaves of *K. candel* directly related to salt tolerance. We suggest that physiological regulation is more important than structural modifications in *K. candel*'s adaptation to saline environments.

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氯化鈉溶液對水筆仔紅樹林幼苗組織構造影響之研究

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本文研究不同鹽度對水筆仔紅樹林幼苗組織構造之影響。在鹽度溶液爲 0 到 550mM NaCl 之範圍內，組織結構之變化明顯的與水筆仔之生長狀態成正相關。水筆仔之生長以在 50 與 100mM NaCl 溶液下爲最好。在此中低鹽度溶液下生長的水筆仔比生長在沒有或高鹽度之植株，有較大之葉面積、較厚之葉片、較大之柵狀組織/葉片厚度比例、較大之根部維管束/根部橫切面面積比例、和根尖細胞中之木質化組織離根尖較遠。本研究並未發現與耐鹽適應機制相關之特化組織或構造。因此在水筆仔耐鹽適應機制中，生理上的調節可能較組織結構之特化重要。

關鍵詞：水筆仔；紅樹林；氯化鈉；解剖構造；鹽度。