

# Variation in the $\delta^{13}\text{C}$ of Two Mangrove Plants is Correlated with Stomatal Response to Salinity

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**Abstract** To better understand the relationship between salinity and the carbon stable isotope composition (expressed as  $\delta^{13}\text{C}$ ) of mangrove plants and to test whether the patterns of variation in  $\delta^{13}\text{C}$  of mangrove plants differ from those of nonhalophytes as response to salinity, the effect of salinity on leaf  $\delta^{13}\text{C}$  in two dominant mangrove species, *Aegiceras corniculatum* and *Kandelia candel*, was studied. Furthermore, to determine whether the variation in  $\delta^{13}\text{C}$  of mangrove species is adjusted by stomatal conductance, *K. candel* was selected as an example, and leaf gas-exchange characteristics of the seedlings were measured. It was observed that both mangrove species had a lower leaf  $\delta^{13}\text{C}$  under their optimum salinity (1.50% for *Ae. corniculatum* and 2.00% for *K. candel*). This variation in  $\delta^{13}\text{C}$  of mangrove plants was attributable largely to stomatal adjustment as for nonhalophytes in which a strong correlation between  $\delta^{13}\text{C}$  and relevant photosynthetic properties is observed. This result suggests that the different response pattern in  $\delta^{13}\text{C}$  was a consequence of the variation in stomata in relation to the different tolerance to salinity. The optimum salinity inferred by leaf  $\delta^{13}\text{C}$  provides a feasible method for comparing salt tolerance between mangrove plants belonging to different species, which is useful for mangrove restoration.

**Keywords** *Aegiceras corniculatum* · Carbon isotope composition ( $\delta^{13}\text{C}$ ) · Halophyte · *Kandelia candel* · Salinity

## Introduction

Salinity is one of the outstanding environmental features of mangrove wetlands. There have been many studies on the ecological characteristics of mangrove plants in response to salinity (for example, Ball and others 1997; Aziz and Khan 2001; Ball 2002; López-Hoffman and others 2006; Lovelock and others 2006; Schmitz and others 2006), but little is known about the correlations between stable carbon isotope composition (expressed as  $\delta^{13}\text{C}$ ) and salinity, even though plant  $\delta^{13}\text{C}$  is seen as an index of the integrated response of a physiologic characteristic to environmental factors (Arslan and others 1999; Poss and others 2000; Choi and others 2005).

The value of  $\delta^{13}\text{C}$  in plant tissues is correlated with their intrinsic salt tolerances, leading to varied patterns of  $\delta^{13}\text{C}$  in different plant species or different parts of a plant. Early studies have shown that halophytic species have a similar pattern of  $\delta^{13}\text{C}$  variation as compared with nonhalophytes, as mentioned in a review by Farquhar and others (1989), and later studies seem to agree with these results (Lin and Sternberg 1992; Goldstein and others 1996; Sobrado 1999; Kao and others 2001; Winter and Holtum 2005).

Farquhar and others (1982) developed a model to describe the relationship between plant  $\delta^{13}\text{C}$  and related parameters:

$$\delta^{13}\text{C}_{\text{plant}} = \delta^{13}\text{C}_{\text{air}} - a - (b - a) C_i/C_a \quad (1)$$

where  $\delta^{13}\text{C}_{\text{plant}}$  and  $\delta^{13}\text{C}_{\text{air}}$  are  $\delta^{13}\text{C}$  of plant and atmospheric  $\text{CO}_2$  (normally 8‰), respectively,  $C_i/C_a$  is the ratio of intercellular to atmospheric  $\text{CO}_2$  concentration,  $a$  and  $b$

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are the carbon isotope discrimination against  $^{13}\text{C}$  during  $\text{CO}_2$  diffusion through stomata (normally 4.4‰) and during  $\text{CO}_2$  fixation by the ribulose-1,5-bisphosphate carboxylase enzyme (normally 27‰), respectively.

As seen from the above model, variation in plant  $\delta^{13}\text{C}$  is associated mainly with stomatal and photosynthetic effects. However, the patterns of  $\text{CO}_2$  exchange properties demonstrate that there are differences between halophytic and nonhalophytic plants as affected by salinity. Increasing salinity commonly induces reduced stomatal conductance ( $g_s$ ), net photosynthetic rate ( $P_n$ ), transpiration rate ( $Tr$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), and other physiologic processes in nonhalophytes such as *Andropogon glomeratus* (Bowman 1987), *Nicotiana tabacum* (Moghaieb and others 2006; Suwa and others 2006), and *Oryza sativa* (Moradi and Ismail 2007). In halophytes, the photosynthetic responses to salinity are complicated. An evergreen perennial halophyte (*Salvadora persica*) exposed to 200 mM NaCl had slightly higher gas exchange properties relative to non-NaCl controls (Maggio and others 2000). Similarly, the maximum  $P_n$  value was 340 mM NaCl in an extreme halophyte, *Sarcocornia fruticosa* (Redondo-Gómez and others 2006). Leaf stomatal conductance and leaf conductance of  $\text{CO}_2$ , which peaked at moderate salinity levels (1.00–2.00%), were detected in three emergent estuarine  $\text{C}_4$  grasses: *Spartina alterniflora*, *S. anglica*, and *S. patens* (Maricle and Lee 2006). However, in other studies, halophytes have shown no photoinhibition stimulated by salinity stresses (Qiu and others 2003).

These contradictory results of the response of photosynthetic properties and plant  $\delta^{13}\text{C}$  to salinity require investigation into whether the patterns of  $\delta^{13}\text{C}$  in mangrove plants differ from nonhalophytes in response to salinity. A particular focus is on the variation of  $\delta^{13}\text{C}$  when plants are grown below the optimum salinity level. In addition, whether the variation in  $\delta^{13}\text{C}$  of mangrove plants is due mainly to stomatal adjustment is explored.

This study examines the effect of salinity on  $\delta^{13}\text{C}$  in two mangrove species, *Ae. corniculatum* and *K. candel*, which are widely distributed in South China. These two mangrove species are generally considered to be typical halophytes. Leaf  $\delta^{13}\text{C}$  from both species and gas exchange properties of *K. candel* seedlings were determined.

## Materials and Methods

Propagules of *Ae. corniculatum* were obtained from the Natural Mangrove Reserve (E117°24'07"-117°30'00"; N23°53'45"-23°56'00") in Zhangjiang Estuary. This region is a typical subtropical ocean monsoon region with a mean annual temperature of 21.2°C and mean annual precipitation of 1714.5 mm. The canopy coverage of *Ae. corniculatum* was 0.9, and mean tree height was 6.0 m (Lin 2001). The collected

propagules were cleaned with distilled water and planted in pots containing beach sand (particle size <3 mm) that was washed with tap water before planting. The pots were about 20 cm in height and 35 cm in diameter. The seedlings were grown in a classroom under natural conditions with a temperature range of 25–35°C during the day and 19–24°C during the night, at natural light:dark photoperiods, relative air humidity of 65–85%, and air [ $\text{CO}_2$ ] of 380–430  $\mu\text{mol mol}^{-1}$ . Seedlings were irrigated with 50% Hoagland nutrient solution and the solutions were changed each week. The full-strength Hoagland nutrient solution contains: 457.19  $\text{mmol m}^{-3}$   $\text{NH}_4\text{NO}_3$ , 138.59  $\text{mmol m}^{-3}$   $\text{MgSO}_4$ , 69.29  $\text{mmol m}^{-3}$   $\text{KH}_2\text{PO}_4$ , 352.97  $\text{mmol m}^{-3}$   $\text{CaCl}_2$ , 353.08  $\text{mmol m}^{-3}$   $\text{KCl}$ , 32.41  $\text{mmol m}^{-3}$   $\text{H}_2\text{BO}_3$ , 6.40  $\text{mmol m}^{-3}$   $\text{MnCl}_2$ , 0.56  $\text{mmol m}^{-3}$   $\text{ZnCl}_2$ , 0.21  $\text{mmol m}^{-3}$   $\text{CuCl}_2$ , 0.07  $\text{mmol m}^{-3}$   $\text{Na}_2\text{MoO}_4$ , 14.56  $\text{mmol m}^{-3}$   $\text{Na}_2\text{HPO}_4$ , and 14.02  $\text{mmol m}^{-3}$   $\text{FeSO}_4$ . The water level was adjusted daily to correct for evaporation. After the second true leaves had fully expanded, the seedlings were irrigated with salt water of differing concentrations (0, 0.50, 1.50, or 2.50% NaCl). Six replicated pots of ten seedlings per pot were used for each treatment and arranged in a completely randomized design. Leaves were sampled at 60 and 130 days following salt solution treatment.

Propagules of *K. candel* were collected from a mangrove wetland in Fugong village, Longhai, Fujian (24°29'N, 117°55'E). The mean annual temperature is 20.9–21.0°C, mean annual precipitation is 1365.1 mm. The mangrove wetlands of *K. candel* were planted in 1962, canopy coverage was 0.9, and mean tree height was 5.5 m (Wang and Lin 2001). The propagules were germinated in sands, irrigated with 50% Hoagland nutrient solution, and grown under the same conditions as *Ae. corniculatum*. After 2 months of growth and once the second true leaves had expanded fully, the plants were irrigated with salt water (0, 0.50, 1.50, 2.00, 2.50, 3.00, or 4.00% NaCl). Six replicated pots of eight seedlings per pot were used for each treatment. The leaves were sampled at 4, 40, and 100 days following treatment with the salt solution.

Leaf samplings from both species were cleaned with distilled water, oven-dried (60°C), and ground to fine powders for  $\delta^{13}\text{C}$  analysis.  $\delta^{13}\text{C}$  analysis was conducted according to the method of Ehleringer and Osmond (1991). The fine powders (5 mg) were combusted at 850°C for 5 h in a vacuum quartz tube. The resulting  $\text{CO}_2$  was purified by adding oxidant and catalyst, and then was introduced into a MAT-253 isotope-mass spectrograph (Thermo Fisher Scientific, Waltham, MA, USA) to measure the ratio of  $^{13}\text{C}/^{12}\text{C}$  compared with Pee Dee Belemnite (0.01124) (Farquhar and others 1989).  $\delta^{13}\text{C}$  was calculated using the following formula:  $\delta^{13}\text{C} (\text{‰}) = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000$ .

For *K. candel*, net photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ), transpiration ( $Tr$ ), and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) were measured after 100 days of salt treatment using a CIRAS-1 Portable Photosynthesis System

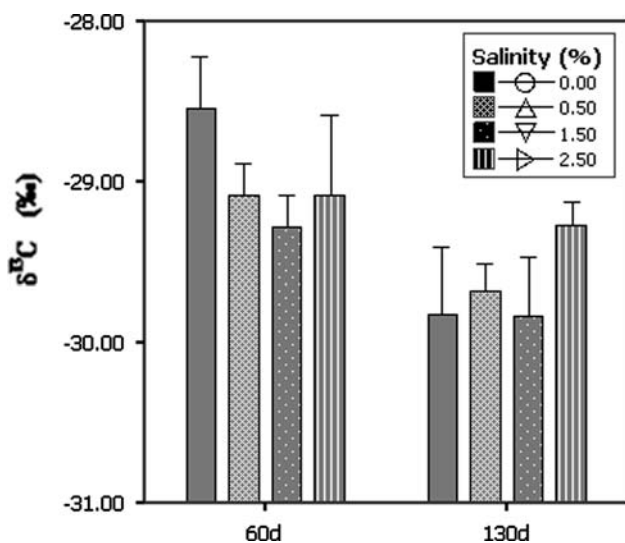
(PP Systems, Haverhill, MA, USA). The leaf temperature was  $35 \pm 0.5^\circ\text{C}$ ,  $\text{CO}_2$  concentration was  $425 \pm 3 \mu\text{mol mol}^{-1}$ , relative humidity was about 70%, and available radiation was greater than  $500 \mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1}$ . Measurements were made on the second or third fully expanded leaves of three seedlings for each treatment. Three leaves were selected from each seedling and about 30 replicated measurements of each leaf were conducted.

Statistical Program for Social Sciences v11.5 (SPSS, Inc., Chicago, IL, USA) was used to analyze the whole data set. The entire data set passed the normality test. For *Ae. corniculatum*, the significance of differences in leaf  $\delta^{13}\text{C}$  among treatments was analyzed with one-way analysis of variance (ANOVA); a paired-samples *t* test was used to analyze the differences in leaf  $\delta^{13}\text{C}$  between 60- and 130-day seedlings. For *K. candel*, a two-way ANOVA was used to determine the significance of differences in leaf  $\delta^{13}\text{C}$  among treatments and sampling time, and the effect of the interaction between salinity and sampling time. For all the significant results ( $p < 0.05$ ), the multiple comparisons were evaluated by the least significant difference (LSD) test. The Pearson correlation test was performed to determine the relationships between  $g_s$  and other photosynthetic parameters of 100-day seedlings of *K. candel*.

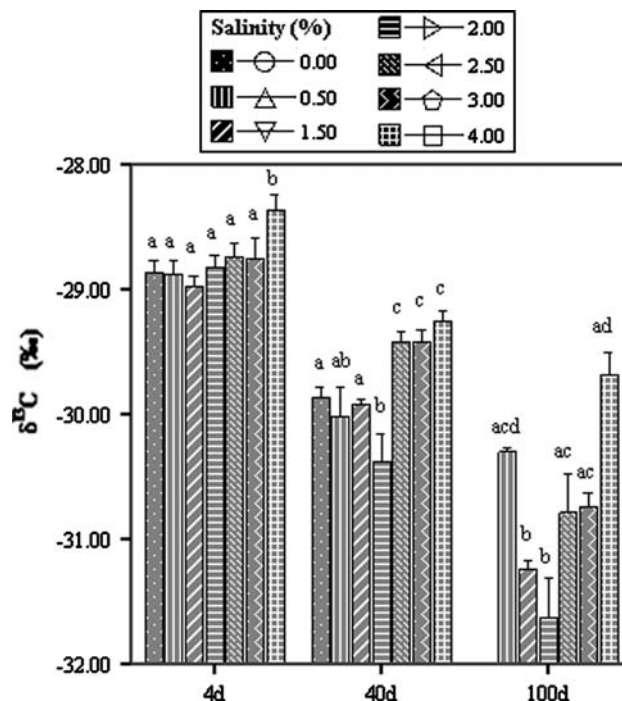
## Results

### Variation in Leaf $\delta^{13}\text{C}$ of Both Species

A similar variation pattern in  $\delta^{13}\text{C}$  values was observed in 60- and 130-dayseedlings of *Ae. corniculatum* (Figure 1).



**Fig. 1** Variation in leaf  $\delta^{13}\text{C}$  of *Ae. corniculatum* seedlings under salinity for 60 and 130 days. There were no significant differences in leaf  $\delta^{13}\text{C}$  between saline treatments in 60-day seedlings or 130-day seedlings. Leaf  $\delta^{13}\text{C}$  in 130-day seedlings is significantly lower than in 60-day seedlings ( $p = 0.034$ )



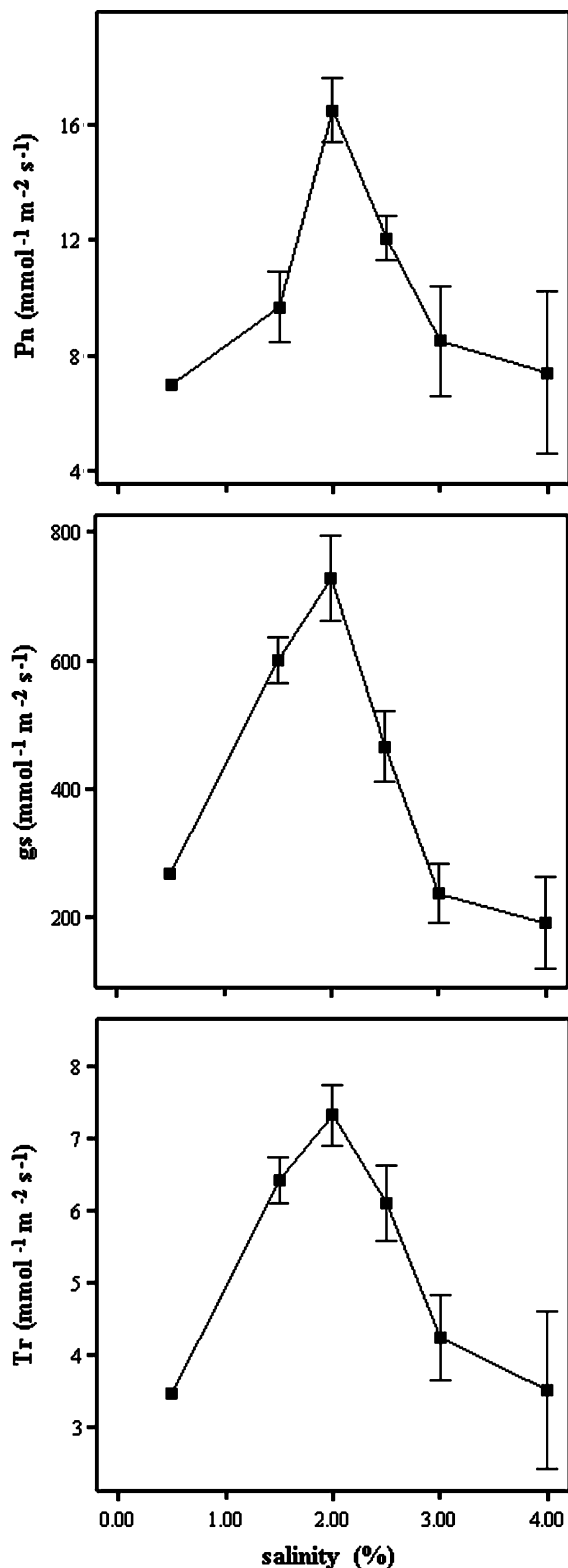
**Fig. 2** Variation in leaf  $\delta^{13}\text{C}$  of *K. candel* seedlings under salinity. Significant differences in leaf  $\delta^{13}\text{C}$  are observed between salinity ( $p < 0.001$  for all seedlings) and between duration of treatments ( $p < 0.001$ )

Leaf  $\delta^{13}\text{C}$  decreased in line with an increase in salinity from 0 to 1.50%, whereas an increased  $\delta^{13}\text{C}$  was observed as the salinity level rose to 2.50%. The values at 60 days were lower than those at 130 days in each treatment ( $p = 0.034$ ).

For *K. candel*, leaf  $\delta^{13}\text{C}$  was unaffected by salinity during the first 4 days of treatment except at the highest level (4.00%). The observed effect was more severe with longer duration of treatment. At 40 days, the lowest value of  $\delta^{13}\text{C}$  was observed at 2.00%, and the value was significantly lower than those at low or high levels of salinity. The pattern of variation in leaf  $\delta^{13}\text{C}$  at 100 days was similar to that at 40 days. The lowest value still occurred at 2.00% (Figure 2). In addition, the values of leaf  $\delta^{13}\text{C}$  in *K. candel* showed a significant reduction with growth stage ( $p < 0.001$ ) similar to the trend observed in *Ae. corniculatum*.

### Photosynthetic Properties

When salinity was below 2.00%,  $P_n$ ,  $g_s$  and  $Tr$  all increased with increasing salinity, whereas further increases in salinity led to decreases in these parameters (Figure 3). A parallel variation in  $P_n$  and  $g_s$  or  $Tr$  indicated that stomatal change is closely associated with variation in  $P_n$ .



**Fig. 3** Variation in  $P_n$ ,  $Tr$ , and  $g_s$  of 100-day seedlings of *K. candel* under salinity.  $P_n$  represents net photosynthetic rate,  $Tr$  represents transpiration rate, and  $g_s$  represents stomatal conductance. There are significant differences in these three parameters among salinity treatments ( $p < 0.001$  for  $P_n$ ,  $g_s$ , and  $Tr$ )

## Discussion

Overall, the results showed that patterns of  $\delta^{13}\text{C}$  variation in *Ae. corniculatum* or *K. candel* in response to salinity differed from the results reported by previous studies on nonhalophytes. In general, elevated salinity leads to a reduction of the discrimination of carbon isotope ( $\Delta$ ) resulting in an increase in leaf  $\delta^{13}\text{C}$ . For example, *Phaseolus vulgaris* and *Gossypium hirsutum* had a reduced value of  $\Delta$  (that is, a higher value of  $\delta^{13}\text{C}$ ) under salinity compared with the control, and the value of  $\Delta$  in the 250-mM treatment was lower than that in the 50-mM NaCl treatment (Brugnoli and Lauteri 1991). In 14 genotype species (*Hordeum vulgare*), the value of  $\Delta$  was significantly lower than the control (Jiang and others 2006). Whether pistachio (*Pistacia vera*) grew in salinity for 30 or 60 days,  $\delta^{13}\text{C}$  in leaf, stem, and root tissues were all lower than the control (Hokmabadi and others 2005). Obviously, a positive relationship between plant  $\delta^{13}\text{C}$  and salinity was observed in these nonhalophytes, and it has been proven in many previous studies (Farquhar and others 1982). However, variation in leaf  $\delta^{13}\text{C}$  of these two mangrove plants in this study had a high-low-high curve in response to salinity. That is, the lowest value of leaf  $\delta^{13}\text{C}$  was at the optimum salinity level.

Why does the variation in  $\delta^{13}\text{C}$  of these two mangrove plants differ from that of nonhalophytes in response to salinity? In terms of the relationship between plant  $\delta^{13}\text{C}$  and photosynthesis described by Farquhar and others (1989), it can be concluded that the values of  $\delta^{13}\text{C}$  were determined mainly by three processes: (1) diffusion of  $\text{CO}_2$  through stomata; (2)  $\text{CO}_2$  assimilation by carboxylase; and (3) metabolism of compounds. For a specific plant, leaf  $\delta^{13}\text{C}$  is closely related to  $P_n$ ,  $g_s$ , and  $C_i/C_a$ . Depression of plant photosynthesis induced by salinity may be attributed to stomatal closure, increase in water-use efficiency, and nonstomatal factors such as damaged cell membranes, reduced mesophyll conductance, and decreased Rubisco activity (Ball and Farquhar 1984; Seemann and Critchley 1985; Sobrado 1999). The dominant factor in depression of photosynthesis will depend on the plant species and its tolerance to salinity. Like  $C_i$ , leaf  $\delta^{13}\text{C}$  could also be used as an indicator for identifying the key limitation factors of photosynthesis (Brugnoli and Lauteri 1991). If stomatal limitation was the main cause of photosynthetic inhibition, leaf  $\delta^{13}\text{C}$  would increase with increasing salinity, whereas if nonstomatal factors became the key limitation, the values would show a decreasing trend.

In this study the strong correlation between  $g_s$  and leaf  $\delta^{13}\text{C}$  and the other related photosynthetic parameters suggested that stomatal adjustment was the key factor influencing leaf  $\delta^{13}\text{C}$  variation (Table 1). Photosynthetic

**Table 1** Correlation between  $g_s$  and Other Photosynthetic Parameters of 100-day Seedling of *K. candel*

	Salinity	$\delta^{13}\text{C}$	$P_n$	$C_i/C_a$	$P_n/g_s$
$g_s$ Pearson correlation	-0.525 <sup>a</sup>	-0.790 <sup>a</sup>	0.668 <sup>a</sup>	-0.533 <sup>a</sup>	-0.709 <sup>a</sup>
Significance	0.002	0.000	0.000	0.002	0.000

<sup>a</sup> Correlation is significant at the 0.01 level (2-tailed)

changes could explain the variation in  $\delta^{13}\text{C}$  of *K. candel*. At salinity below 2.00%, increasing  $g_s$  led to stronger discrimination against  $^{13}\text{C}$ , which resulted in a lower  $\delta^{13}\text{C}$  in leaves. As salinity increased beyond this concentration,  $g_s$  decreased and the reduction of discrimination against  $^{13}\text{C}$  led to higher  $\delta^{13}\text{C}$ . It is noted that the reduction in leaf  $\delta^{13}\text{C}$  with increasing salinity observed at salinity levels below 2.00% was not caused by variation of nonstomatal factors but by stomatal closure. Therefore, the difference in response patterns of leaf  $\delta^{13}\text{C}$  between mangrove plants and nonhalophytes is because these species have different stomatal responses to salinity. For example, in three nonhalophytes, *Myrica cerifera*, *Pinus taeda*, and *Baccharis halimifolia*, stomatal conductances were significantly reduced when irrigated by salt solutions (Tolliver and others 1997). However, halophyte species often grow well under moderate salinity. Winter and Holtum (2005) have proposed that nonsalinity is not the optimum condition for halophyte species, with nonsalinity proving a stress factor like high salt levels. For *K. candel* seedlings in this study, salinity of about 2.00% could be considered the optimum growth condition. Other studies also demonstrated that *K. candel* is suitable for growing in a saline environment. For example, *K. candel* seedlings grown in 50 and 100 mM NaCl had greater leaf area and thickness, higher ratios of palisade to leaf thickness, and higher stele to root cross-section-area proportions (Hwang and Chen 1995). Hwang and Chen (2001) continued to study photosynthesis in *K. candel* and showed that photosynthesis and dry weight was higher at 5.00% than at 0 or 2.00% salinity. Another study on *K. candel* showed that suitable salinity ranged from 0.83 to 1.65% (Yang 1996). Although the optimum salinity reported in previous studies was inconsistent, these results all indicated that *K. candel* was suitable for growing under saline conditions. This phenomenon was also observed in other mangrove species. *Avicennia germinans* was cultivated in 0, 1.00, 2.50, 4.00, and 5.50% salinity and  $P_n$ ,  $g_s$ , and  $C_i$  reached maximum values at a salinity level of 1.00% (Suárez and Medina 2006).  $P_n$  of *Bruguiera parviflora* grown at 100 mM was higher than at nonsalinity and other saline levels (Parida and others 2004). Fv/Fm in seedlings of *Ae. corniculatum* and *Avicennia marina* was greatest at 25% seawater and

less in lower and higher salinities (Christian 2005). In a field experiment it was shown that *Av. marina* had greater values of mean  $\text{CO}_2$  exchange, conductance, and transpiration in a high-salinity site at Durban Bay (3.50%) than in a lower-salinity site at Beachwood (<1.20%) (Naidoo and others 2002).

Although the photosynthetic characteristic of *Ae. corniculatum* was not determined in this study, many previous studies have shown that the maximum rate of photosynthesis of this species also occurred at a suitable salinity. In *Ae. corniculatum* grown in 0, 5, 25, 50, 75 and 100% seawater, seedlings at 25% seawater had the greatest photon efficiency of PSII and total leaf chlorophyll per unit leaf area (Christian 2005). At 50 mol L<sup>-1</sup> NaCl, the  $\text{CO}_2$  assimilation rate of *Ae. corniculatum* was higher than at 500 mol L<sup>-1</sup> NaCl, and the variation in leaf  $g_s$ ,  $Tr$ , and  $C_i$  paralleled the  $\text{CO}_2$  assimilation rate (Naidoo and von Willert 1995).

However, some previous studies have shown that mangrove species have the same trends in leaf  $\delta^{13}\text{C}$  variation as some nonhalophytes. We noted that these studies lacked data on nonsalinity, such as the study on *Scaevola sericea* (Goldstein and others 1996), *K. candel* (Hwang and Chen 1995, 2001), and *Rhizophora mangle* (Lin and Sternberg 1992). The results of these cases were not sufficient to reflect the patterns of  $\delta^{13}\text{C}$  variation response to salinity. To our knowledge, only one study on variation in  $\delta^{13}\text{C}$  of cellulose in a mangrove species showed a pattern similar to our results. When *Avicennia germinans* was cultivated in 18 and 45% seawater, the lowest  $\delta^{13}\text{C}$  in cellulose was detected in 18% seawater treatment, which was lower than in 0 and 45% seawater. But this variation pattern was not observed by Ish-Shalom-Gordon and others (1992), who reported that variation in cellulose  $\delta^{13}\text{C}$  was not related to salinity.

Decreases in  $\delta^{13}\text{C}$  with growth stage in *Ae. corniculatum* and *K. candel* have also been observed in other species. Different genotypes of rice grown at 1 and 8 dS m<sup>-1</sup> salinity did not exhibit a significant correlation between leaf  $\delta^{13}\text{C}$  and salinity at 25 days of salt solution treatment. Although at 33 days,  $\Delta$  showed a significant negative linear correlation with salinity (Poss and others 2004). Several factors may explain the reduction of plant  $\delta^{13}\text{C}$  with growth stage. Enhanced respiration may be one of the factors because  $\text{CO}_2$  released from respiration has more  $^{13}\text{C}$  (Damesin and Lelarge 2003). Terwilliger and others (2001) found that the values of  $\delta^{13}\text{C}$  in soluble sugar and the content of phosphorus enolpyruvic acid carboxylate enzyme (PEPCase) were different between new and old leaves. Enrichment of  $^{13}\text{C}$  in new leaves was the result of sucrose synthesis after export from old leaves. The higher content of PEPCase in new leaves made the discrimination against  $^{13}\text{C}$  during  $\text{CO}_2$  fixation more than normal values.

In conclusion, the response patterns of leaf  $\delta^{13}\text{C}$  to salinity in *Ae. corniculatum* and *K. candel* differ from nonhalophytic species, but the factor that determined the variation of leaf  $\delta^{13}\text{C}$  in mangrove plants was still attributable to stomatal adjustment. The difference in response patterns of leaf  $\delta^{13}\text{C}$  between mangrove plants and nonhalophyte species was a consequence of the different stomatal response to salinity that was a result of their different salinity tolerances. At relatively moderate salinity (1.50‰ for *Ae. corniculatum* and 2.00‰ for *K. candel*), leaves have the lowest  $\delta^{13}\text{C}$  values, which provides a feasible method for selecting salt-tolerant species, especially for comparing plants belonging to different species. The difference may be very useful in mangrove restoration because salinity is closely related to mangrove distribution in the coastal and estuary regions.

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