Growth and physiological responses of two mangrove species (Bruguiera gymnorrhiza and Kandelia candel) to waterlogging

Y. Ye a,b, Nora F.Y. Tam a,*, Y.S. Wong a, C.Y. Lu b

a Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong
b Environmental Science Research Centre, Xiamen University, Xiamen, Fujian, People’s Republic of China

Received 21 May 2002; received in revised form 9 October 2002; accepted 9 October 2002

Abstract

Effects of duration of waterlogging on growth and physiological responses of two mangrove species, Bruguiera gymnorrhiza and Kandelia candel, were investigated. The relative growth rate of B. gymnorrhiza decreased significantly with waterlogged time, with the highest value found for drained plants and the lowest in plants under 12 weeks waterlogging. On the contrary, no significant difference was found between waterlogged and drained K. candel plants. The shoot to root biomass ratio of K. candel increased when subjected to 8 or 12 weeks waterlogging but little change was recorded in B. gymnorrhiza, indicating a shift in biomass allocation from roots to shoots in K. candel under prolonged waterlogging but not in B. gymnorrhiza. These different growth responses between the two mangrove species supported the hypothesis that K. candel is more tolerant to waterlogging than B. gymnorrhiza. Under 12 weeks waterlogged treatment, root oxidase activity significantly decreased in B. gymnorrhiza but increased in K. candel. Chlorophyll contents of K. candel increased more rapidly in response to waterlogging than B. gymnorrhiza. Activities of both peroxidase and superoxide dismutase increased significantly in leaves of K. candel when the waterlogging period was longer than 8 weeks, while only the peroxidase activity of B. gymnorrhiza showed a significant increase, indicating that K. candel had stronger resistance to the oxidant damage resulting from waterlogging. These physiological indicators further supported the hypothesis that K. candel is more tolerant to waterlogging than B. gymnorrhiza.

Keywords: Biomass partitioning; Nitrate reductase; Peroxidase; Relative growth rate; Root oxidase activity; Superoxide dismutase

1. Introduction

Mangroves, distributed in intertidal zones of tropical and subtropical coastlines, are facing challenges from global sea level rise (Ellison and Farnsworth, 1997). In recent years, there is also a potential of using mangroves as natural wetlands for treating wastewater (Wong et al., 1997). Both sea level rise and wastewater discharge will prolong the waterlogged time for mangroves and affect their growth and physiology. Mangroves are considered waterlogging-tolerant (McKevin et

* Corresponding author. Tel.: +852-2788-7793; fax: +852-2788-7406.
E-mail address: bhntam@cityu.edu.hk (N.F.Y. Tam).
al., 1998). Three species of the western mangrove group, *Rhizophora mangle*, *Avicennia germinans* and *Laguncularia racemosa*, have been studied on changes of photosynthetic rate, water relation, and root respiration in response to waterlogging or hypoxia (Pezeshki et al., 1997; McKee, 1996; Ellison and Farnsworth, 1997). Naidoo (1985) examined the effects of waterlogging on chlorophyll, tissue water potential and ion concentration of three mangrove species of the eastern group, *Avicennia marina*, *Rhizophora mucronata* and *Bruguiera gymnorrhiza*. The research work on several mangrove species, namely *Aegiceras corniculatum*, *Avicennia marina*, *B. gymnorrhiza*, and *Rhizophora stylosa*, of the eastern group suggested that mangrove seedlings especially the viviparous ones showed a significant decrease in photosynthetic gas exchange and CO₂ assimilation in response to root anoxia (Youssef and Saenger, 1998). This research group also found that the differential tolerance of mangrove seedlings to waterlogging was not simply based on their ability to oxidase the rhizosphere, the plants would develop anatomical and morphological adaptations to flooding by which roots conserve oxygen to maintain aerobic metabolism for longer periods during submergence (Youssef and Saenger, 1996). Other studies on non-mangrove species have shown that waterlogging stress would affect N metabolism including nitrate reductase activity (Ernst, 1990; Sung, 1993), and the removal of active oxygen by peroxidase and superoxide dismutase (Yan et al., 1995). Takemura et al. (2000) also reported that *B. gymnorrhiza* responded to salt stress by a steep increase in superoxide dismutase activity as active oxygen species including superoxide can be induced by various environmental stresses. These research suggests that physiological responses are good parameters indicating the degree of plant tolerance to waterlogging.

*B. gymnorrhiza* and *Kandelia candel* are two major mangrove species of the eastern group and are dominant along South China coastlines. These two species are found in the provinces of Hainan, Guangdong (including Hong Kong and Macau), Guangxi and Fujian, China (Li and Lee, 1997). In Hong Kong, *K. candel* is found in all mangrove swamps while *B. gymnorrhiza* colonizes 28 out of 44 mangrove swamps, and both species are considered as dominant species (Tam et al., 1997). However, the differences in responses and tolerance to prolonged waterlogging time have not been well known between these two species. We hypothesize that *K. candel* has stronger tolerance to waterlogging than *B. gymnorrhiza* because the former species seems to occupy the tidal zone lower than the latter one in Hainan, Fujian and Guangdong Provinces as reported by Lin (1999). Tam and Wong (2000) also observed that *B. gymnorrhiza* is a characteristic species in the middle mangrove community but also extends into the transitional landward communities while *K. candel* can be found in different tidal positions (from landwards to seawards) and has some pioneering propensity in Hong Kong. In addition, we attempt to test this hypothesis from different growth and physiological responses of the two species under various waterlogging time. Differences in biomass partitioning and relative growth rate (RGR) between treatment times of the same species were used to indicate growth response under waterlogging. These two parameters have been considered as important waterlogging indicators in other mangrove species (Naidoo, 1985; Pezeshki et al., 1997; Ellison and Farnsworth, 1997) and non-mangrove species (Naidoo and Naidoo, 1992). The concentrations of various photosynthetic pigments in leaves, root oxidase activity, nitrate reductase activity, peroxidase activity and superoxide dismutase activity were determined to explore the effects of waterlogging on various physiological processes, including photosynthesis, root respiration, nutrient utilization and anti-oxidation.

2. Materials and methods

2.1. Materials

Mature propagules of *K. candel* (L.) Druce and *B. gymnorrhiza* (L.) Lam. were collected at Mai Po Mangrove Nature Reserve (114°05'E, 22°32'N) in Hong Kong in March and April 1997, respectively. Four propagules were planted in a plastic pot...
containing 4 kg of soil collected from the same mangrove forest. These plants were kept in a greenhouse with temperature of 25±5 °C and light intensity of 480 μmol m⁻² s⁻¹ from natural sunlight. Each pot was irrigated with 400 ml artificial seawater with a salinity of 15 g l⁻¹ (prepared by dissolving a commercial salt purchased from Instant Ocean, Aquarium Systems, Inc., Mentor, Ohio) every 2 days from March to October for the propagules to establish. Each pot (18 cm in diameter and 20 cm in height) had six draining holes (0.6 cm in diameter) at the bottom so water was able to drain freely by gravitational force. The propagules were allowed to germinate and grow for 7 months before the experiment.

2.2. Experimental design

For each species, four treatments, each with four replicates in a complete randomized design, were set up to examine the growth and physiological responses to four waterlogged periods, that is, drained for 12 weeks (D12W0), drained for 8 weeks and then waterlogged for 4 weeks (D8W4), drained for 4 weeks and then waterlogged for 8 weeks (D4W8), and waterlogged for 12 weeks (D0W12). During drained periods, each pot was put onto a shallow tray (3 cm deep) and irrigated with 400 ml artificial seawater (salinity of 15 g l⁻¹) every 2 days, in the same way as previously described. For waterlogged treatments, each pot was placed inside a plastic container (30 cm long × 40 cm wide × 30 cm high) full of artificial seawater (salinity of 15 g l⁻¹) to ensure that the pot was waterlogged and the soil surface was inundated with 5 cm seawater. The water level of each container was adjusted daily with tap water to compensate the amount of water lost by evaporation. Seawater in each container was replaced by freshly prepared seawater weekly to make sure that seawater would not get stale. At the end of the waterlogging treatments, all plants were harvested, and growth and physiological parameters were determined.

2.3. Soil redox potential and plant growth analysis

At the end of the experiment, soil redox potentials of each pot were measured at three random points by inserting the platinum electrode (Combination Redox Probe made in TPS Pty. Ltd., Brisbane, Australia) into the soil to a depth of 5 cm. The electrode was allowed to equilibrate for 30 min before taking the readings. At the beginning and at the end of the experiment, stem basal diameter (D) at the first stem node (i.e. the oldest node of the stem) and stem height (H) (excluding hypocotyl) of each plant were measured. Biomass partitioning was determined by separating each individual plant into shoot (leaf and stem) and root portions, washed and dried at 105 °C, the dry weight was then measured. Hypocotyl was excluded during either shoot or root biomass measurement because the weight of hypocotyl did not change during the experiment and also because its heavy weight would affect the accuracy of other biomass measurements. Data on total biomass (B), stem basal diameter (D) and stem height (H) of each plant of the control at the end of the experiment were used to fit the non-destructive allometric equations suggested by C荺ron and Novelli (1984). The fit equations for these two species were given as follows:

\[
\log B = 0.6439 + 0.3053 \log(D^2H) \\
(n = 16, \ P < 0.05)
\]

\[
\log B = 0.3353 + 0.5111 \log(D^2H) \\
(n = 16, \ P < 0.05)
\]

The initial biomass values were then estimated using these equations. The RGR was calculated as:
Here $B_1$ and $B_2$ are total biomass at the beginning ($t_2$) and end ($t_1$) of the experimental period (Hunt, 1978).

2.4. Physiological analysis

2.4.1. Root oxidase activity

The method used to determine root oxidase activity, in terms of $\alpha$-naphthylamine oxidized, was similar to that described by Ota (1970), Zhang (1990) and further modified by Satpathy et al. (1997). The fine roots from one randomly selected plant in each of the four replicates for each treatment were harvested. About 0.2 g fresh and tapwater-washed fine root was immersed in 20 ml solution (a 1:1 mixture of 50 $\mu$g ml$^{-1}$ $\alpha$-naphthylamine and 0.1 mol l$^{-1}$ phosphate buffer at pH 7.0) and kept at 25 °C for 24 h incubation. At the beginning and at the end of the incubation, 0.2 ml of the solution was added to 2 ml deionized water, 0.1 ml 1% sulfanilic acid (w/v in 30% acetic acid) and 0.1 ml 0.01% sodium nitrite. The mixture was kept at room temperature for 20 min and the absorbance at 510 nm was then measured. Root oxidase activity was determined by the decrease of $\alpha$-naphthylamine during the incubation period and expressed as ng $\alpha$-naphthylamine h$^{-1}$ mg$^{-1}$ DW.

2.4.2. Contents of pigments in leaves

For all pigment and enzyme assays of leaf samples, the third pair of leaves from the top of one randomly selected plant in each pot of the four replicates for each treatment was used as they were considered mature and had similar development time. About 0.1 g fresh leaf tissues was ground in cold mortar with 10 ml 80% acetone. The homogenate was centrifuged at 10000 $\times$ g for 3 min. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid were determined by Lichtenthaler and Wellburn (1983) method.

2.4.3. Nitrate reductase activity

About 0.3 g fresh plant tissues of leaf, the youngest inter-node (i.e. the portion of stem from the shoot apex to where the first pair of leaves grows), and root tip from one randomly selected plant in each of the four replicates for each treatment were ground in 1.5 ml cold 0.1 mol l$^{-1}$ phosphate buffer (pH 7.5) containing 1 mmol l$^{-1}$ Na$_2$EDTA. The completely homogenized mixture was centrifuged at 14000 $\times$ g for 20 min and the supernatant, the extract of nitrate reductase, was kept at 4 °C. The enzyme activity was then measured according to Ross (1974) with some modification. 0.2 ml enzyme extract was well mixed with 0.2 ml 0.28% NADH (w/v in deionized water) and 1.6 ml buffered nitrate solution, and kept at 30 °C for 24 h. At the end of incubation, the reaction was stopped by adding 1 ml 1% sulfanilamide (w/v in 1.5 N HCl) and 1 ml 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (w/v in deionized water). The absorbance at 540 nm was measured to determine the nitrite production. Nitrate reductase activity was expressed as nitrite producing rate.

2.4.4. Activities of peroxidase and superoxide dismutase

The methods used for peroxidase and superoxide dismutase extraction and activity determination were similar to those of Putter (1974), Liu and Zhang (1994), with some modifications. Portions of fresh plant materials collected for determination of nitrate reductase activity were ground and homogenized with five times of 62.5 mmol l$^{-1}$ phosphate buffer (pH 7.8) in an ice bath in the presence of polyvinyl pyrrolidone to neutralize the effect of phenol of plant tissues. The homogenate was centrifuged at 1500 $\times$ g and 4 °C for 20 min. The supernatant, the extract of peroxidase and superoxide dismutase, was stored at 4 °C before determination.

For peroxidase activity measurement, 20 $\mu$l of the enzyme extract was diluted to 1 ml with deionized water, and mixed with 3 ml combined reagent (consists of 500 ml of 0.1 mol l$^{-1}$ phosphate buffer, 280 $\mu$l guaiacol and 190 $\mu$l hydrogen peroxide). The absorbance at 470 nm was measured at 1 min interval for 5 min. An
increase of 0.01 absorbance per minute was equaled to one unit of peroxidase activity.

Superoxide dismutase activity was measured in terms of degree of inhibition to photo-reduction of nitro blue tetrazolium (NBT). The following reagents in a sequence of 2.4 ml 62.5 mmol l\(^{-1}\) phosphate buffer, 0.2 ml 0.06 mmol l\(^{-1}\) riboflavin, 0.2 ml 30 mmol l\(^{-1}\) methionine, 0.1 ml 0.003 mmol\(^{-1}\) Na\(_2\)EDTA, 20 \(\mu l\) enzyme extract, and 0.2 ml 1.125 mmol l\(^{-1}\) NBT were mixed. The enzyme solution was substituted by the buffer solution to determine the maximum photo-reduction of NBT. The reaction was carried out under illumination (80 \(\mu m\)ol m\(^{-2}\) s\(^{-1}\)) for 1 h, and the absorbance at 560 nm was measured. An enzyme unit was calculated as inhibition of maximum photo-reduction of NBT by 50%.

2.5. Data analysis

Mean and standard deviation (S.D.) values of four replicates were calculated. Two-way ANOVA with species and waterlogged treatments as the factors were employed to test any difference between two mangrove species, among waterlogged treatments, and interactions between species and treatments. If a significant difference was found in either the species factor or the interaction term, the waterlogged treatment effect on each species would be tested again using one-way ANOVA. The Student–Newman–Keuls multiple comparison method was used if any significant difference was found among treatments.

3. Results

3.1. Growth response

At the beginning of the experiment, all pots had similar redox potentials as the soils were drained before. The soil redox potentials of *B. gymnorrhiza* pots and *K. candel* pots were 230 ± 2 and 236 ± 1 mV (\(n = 16\)), respectively. At the end of the experiment, soil redox potentials decreased significantly with waterlogged time, in the descending order of D12W0 > D8W4 > D4W8 > D0W12 for both *B. gymnorrhiza* (\(P < 4 \times 10^{-5}\)) and *K. candel* pots (\(P < 8 \times 10^{-8}\)) (Fig. 1).

The final shoot biomass of *B. gymnorrhiza* was significantly higher than that of *K. candel* but there was no significant difference in the final root biomass between the two species (Fig. 2 and Table 1). Growth responses, in terms of biomass partitioning and RGR, to waterlogging were different between the two species. *K. candel* in general had lower shoot/root biomass ratio (S/R) than *B. gymnorrhiza*. For *B. gymnorrhiza*, no significant differences were found in S/R among the four treatments but S/R of *K. candel* was significantly lower in drained (D12W0) plants when compared with plants subjected to 8 weeks (D4W8) or 12 weeks (D0W12) waterlogging.

The RGR was not significantly different among four treatments in *K. candel* but the RGR dropped significantly with waterlogged time in *B. gymnorrhiza* (Fig. 2). The RGR of *B. gymnorrhiza* was higher than that of *K. candel* if the plants were drained or were waterlogged for less than 8 weeks. However, in the 12 weeks waterlogged treatment (D0W12), there was no significant difference in RGR between *K. candel* and *B. gymnorrhiza*. According to two-way ANOVA, a significant interaction factor was found between species and
3.2. Physiological response

The interaction between species and treatment in terms of root oxidase activity measurement was significant according to two-way ANOVA (Table 1), indicating the two species had different responses to waterlogging. Root oxidase activity of *B. gymnorrhiza* subjected to 12 weeks waterlogging (D0W12) declined significantly when compared with the drained (D12W0), D8W4 and D4W8 plants (Fig. 3). On the contrary, the highest root oxidase activity was found in *K. candel* plants with prolonged waterlogging.

Changes in photosynthetic pigments (chlorophyll and carotenoid) with waterlogged time are shown in Fig. 4. For *B. gymnorrhiza*, D0W12 treated plants had significantly higher concentrations of chlorophyll a, chlorophyll b and total chlorophyll than the other treatments (Fig. 4). However, for *K. candel*, both D0W12 and D4W8 treatments resulted in significantly higher chlorophyll contents than the control, and in terms of chlorophyll a contents, even the D8W4 treatment showed a significantly higher value than the control. For both species, total carotenoid contents were highest for D0W12 treatments and the values between other treatments were not significantly different.

Both species had higher nitrate reductase activity in leaf and stem than in root (Fig. 5). Interaction between species and treatment was very significant in root and stem but not so in leaf (Table 1), indicating that *B. gymnorrhiza* was different from *K. candel* in responding to waterlogging by means of root and stem nitrate reductase activity. Although nitrate reductase activity in leaf and stem of both species tended to increase with waterlogged time, a significantly higher stem nitrate reductase than the control was found when *B. gymnorrhiza* was waterlogged for 8 weeks (D4W8) while such difference was only observed in 12 weeks waterlogged *K. candel* pots (D0W12). The highest root nitrate reductase activity was found in D0W12 treated *B. gymnorrhiza* but for *K. candel*, all waterlogged treatments had significantly lower values than control. For each component of each species significant differ-

---

**Fig. 2.** Final biomass of shoot and root, shoot/root biomass ratio, and relative growth rate (RGR) of *B. gymnorrhiza* (filled column) and *K. candel* (open column) at the end of different waterlogging treatments. Mean and S.D. of four replicates are shown, and mean values with the same letter were not significantly different at $P \leq 0.05$ level according to one-way ANOVA test. D12W0, D8W4, D4W8 and D0W12 represent drained for 12 weeks, drained for 8 weeks and then waterlogged for 4 weeks, drained for 4 weeks and then waterlogged for 8 weeks, and waterlogged for 12 weeks, respectively.
ences in nitrate reductase activity were found among four treatments (Table 1).

The peroxidase activities in *K. candel* were significantly higher than in *B. gymnorrhiza* (Fig. 6 and Table 1). For *K. candel*, the activity had highest value in leaf of D4W8 treatment but changed very little in stem and root when subjected to waterlogging. In leaf and root of *B. gymnorrhiza*, peroxidase activities were significantly different among treatments and were higher when they were waterlogged for 12 weeks. No significant differences were found among all treatments in stem for both species (Table 1).

Both *B. gymnorrhiza* and *K. candel* had similar patterns of stem and root superoxide dismutase activities in response to waterlogging, with highest

In Table 1, the results of ANOVA on shoot biomass, root biomass, biomass ratio of shoot/root (S/R), root oxidase activity, leaf pigment contents including chlorophyll (chl.) and carotenoid (car.), and activities of nitrate reductase, peroxidase and superoxide dismutase of leaf, stem and root of *B. gymnorrhiza* (*Bg*) and *K. candel* (*Kc*) seedlings are presented.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Sources of variation for 2-way ANOVA</th>
<th>1-way ANOVA: Treatment effects on individual species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species (S) Treatment (T) S × T</td>
<td><em>Bg</em></td>
</tr>
<tr>
<td>Shoot biomass</td>
<td>11.98** 1.30 0.32</td>
<td>1.09</td>
</tr>
<tr>
<td>Root biomass</td>
<td>1.89 1.46 1.75</td>
<td>3.99</td>
</tr>
<tr>
<td>S/R</td>
<td>91.65*** 0.48 4.85*</td>
<td>3.38</td>
</tr>
<tr>
<td>RGR</td>
<td>28.01*** 12.36*** 9.62***</td>
<td>144.8***</td>
</tr>
<tr>
<td>Root oxidase activity</td>
<td>99.1*** 5.41** 36.66***</td>
<td>16.11***</td>
</tr>
<tr>
<td>Pigments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl.a</td>
<td>35.16*** 66.87*** 29.25***</td>
<td>27.88***</td>
</tr>
<tr>
<td>Chl.b</td>
<td>6.57* 49.82*** 27.04***</td>
<td>36.48***</td>
</tr>
<tr>
<td>Total chl.</td>
<td>25.99*** 64.08*** 29.62***</td>
<td>30.64***</td>
</tr>
<tr>
<td>Total car</td>
<td>0.31 22.89*** 0.51</td>
<td>8.86**</td>
</tr>
<tr>
<td>Nitrate reductase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>12.47** 12.07*** 2.6</td>
<td>4.48*</td>
</tr>
<tr>
<td>Stem</td>
<td>10.77** 33.74*** 7.64***</td>
<td>39.83***</td>
</tr>
<tr>
<td>Root</td>
<td>8.99** 18.82*** 28.11***</td>
<td>28.72***</td>
</tr>
<tr>
<td>Peroxidase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>54.68*** 8.08** 9.41***</td>
<td>6.96*</td>
</tr>
<tr>
<td>Stem</td>
<td>98.44*** 3.66* 2.23</td>
<td>2.73</td>
</tr>
<tr>
<td>Root</td>
<td>136.19*** 3.37* 2.02</td>
<td>9.82**</td>
</tr>
<tr>
<td>Superoxide dismutase activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>8.19* 1.46 6.43**</td>
<td>1.65</td>
</tr>
<tr>
<td>Stem</td>
<td>110.49*** 14.68*** 1.78</td>
<td>9.31***</td>
</tr>
<tr>
<td>Root</td>
<td>41.23*** 20.96*** 1.06</td>
<td>15.07***</td>
</tr>
</tbody>
</table>

*F*-values are given and significant effects are denoted as: *, 0.05; **, 0.01 and ***, 0.001 probability levels.

4. Discussion

4.1. Growth and biomass partitioning response to waterlogging

At the end of the experiment, soil redox potentials at a depth of about 5 cm decreased significantly with waterlogged time, indicating that...
continuous waterlogging resulted in oxygen-deficiency in mangrove soils. When harvesting the roots and soils from each pot, it is clear that soils in the bottom layer of the prolonged waterlogging pots were much blacker and more anoxic than that in the control. The redox potential data in the bottom soil layer was not measured because it was very difficult to insert the redox probe into the deep layer. Soil redox potentials of *K. candel* pots were higher than those in *B. gymnorrhiza* pots (Fig. 1) although there was no significant difference in root biomass between the two species (Fig. 2), suggesting that roots of *K. candel* may transport more oxygen into soil than those of *B. gymnorrhiza*. Therefore, it seems that *K. candel* might have more tolerance to waterlogging than *B. gymnorrhiza*. Youssef and Saenger (1996) reported that viviparous mangroves had differential tolerance to waterlogging and one strategy to protect the plants was related to morphological and anatomical adaptations by which roots conserve oxygen to maintain aerobic metabolism for longer periods during submergence.

Different growth responses to waterlogging between *B. gymnorrhiza* and *K. candel* indicate that these two mangrove species had different tolerance to waterlogging. The RGR of *B. gymnor-
norrhiza decreased significantly with duration of waterlogging but no significant difference was found between waterlogged and drained K. candel plants (Fig. 2). This partly confirmed our hypothesis that K. candel is more tolerant to waterlogging than B. gymnorrhiza.

K. candel had lower shoot/root biomass ratio (S/R) than B. gymnorrhiza, indicating that the species more tolerant to waterlogging would allocate more biomass to roots. This is similar to the conclusion by Pezeshki et al. (1997) that Avicennia germinans, a species relatively more sensitive to low redox potentials, had lower root weight ratio (that is higher shoot/root biomass ratio) than the more flood-tolerant mangrove species, R. mangle.

The shoot/root biomass ratio varies with changing conditions of internal and external plant environment, and the change is an adaptive
character of many plant species (Koler and Kozinka, 1992). The biomass ratio in both *B. gymnorrhiza* and *K. candel* subjected to 4 weeks waterlogging (D8W4) was not significantly different from that of the drained (D12W0) plants (Fig. 2), indicating that these two mangrove species had a certain degree of tolerance to waterlogging. However, when the waterlogging lasted longer than 8 weeks, significant increases in shoot/root biomass ratio were found in *K. candel*, implying a shift in its biomass allocation from root to shoot under prolonged waterlogging. From this, we concluded that biomass shift from root to shoot is an adaptation to prolonged waterlogging time for mangrove species more tolerant to waterlogging.

Prolonged waterlogging may have two effects on plants: (1) increase in water availability that will benefit plant growth, and (2) deficit in oxygen supply that will inhibit plant growth. For a species more tolerant to waterlogging, the enhancement of available water will out-compete the inhibition of oxygen deficit after waterlogged for some time, and less root biomass will be necessary to absorb water from the root environment. However, will this reduce root nutrient uptake from soil? Rubio et al. (1997) hypothesized that reduction of biomass allocation to roots for waterlogging-tolerant species will enhance the uptake of nutrient per unit of root biomass under waterlogging. They partially tested it by the P uptake by *Paspalum dilatatum* and considered that this advantage would be one of the reasons for the increased relative abundance of *P. dilatatum* in the community after waterlogging periods. Therefore, under prolonged waterlogging biomass shift from root to shoot (that is increase in S/R) for *K. candel* may maintain its RGR through the increase in nutrient uptake per unit of root biomass.

4.2. Physiological response to waterlogging

McKee (1996) showed that root zone hypoxia led to significant decreases in respiration rates in intact root systems of *Avicennia germinans* and *L. racemosa*, the two relatively sensitive species to waterlogging, compared to *R. mangle*, a more waterlogging-tolerant mangrove species. The root respiration rates could be reflected by the root oxidase activity to α-naphthylamine (Zhang, 1990). There was little difference in root oxidase activity between the drained (D12W0), 4 weeks waterlogged (D8W4) and 8 weeks waterlogged (D4W8) treatments in both *B. gymnorrhiza* and *K. candel* (Fig. 3), showing that these two species were able to tolerate a short duration of water-
logging. However, different responses in root oxidase activity to waterlogging occurred between \textit{B. gymnorrhiza} and \textit{K. candel} under 12 weeks waterlogged treatment, with a significant decrease in \textit{B. gymnorrhiza} but an increase in \textit{K. candel}. This also supports the hypothesis that \textit{K. candel} is more tolerant to waterlogging than \textit{B. gymnorrhiza}.

Different species responded to waterlogging differently in contents of leaf photosynthetic activity. Previous studies showed that chlorophyll contents and/or photosynthesis decreased with waterlogging or under flooding stress (Ashraf and Yasmin, 1991; Huang et al., 1995; McKevlin et al., 1995). However, chlorophyll content of marshhay cordgrass was not affected by hypoxia (Pezeshki et al., 1993). Naidoo and Naidoo (1992) found that carbon dioxide assimilation rates of \textit{Sporobolus virginicus} were higher in well-watered and waterlogged treatments than in drained ones, and suggested that this species was tolerant to waterlogging. Similarly, leaf chlorophyll content of \textit{R. mangle} increased when the sea level in simulated tidal tanks had a rise of 16 cm (Ellison and Farnsworth, 1997). Chlorophyll and carotenoid concentrations of the 12 weeks waterlogged \textit{B. gymnorrhiza} and \textit{K. candel} were higher than the drained plants (Fig. 4). The chlorophyll contents of 8 and 12 weeks waterlogged \textit{K. candel} were significantly higher than the control but such increase was only found in 12 weeks waterlogged \textit{B. gymnorrhiza}. This again indicates that \textit{K. candel} could enhance the synthesis of chlorophyll, a very important photosynthetic pigment, more rapidly in response to waterlogging than \textit{B. gymnorrhiza}, and provides further evidence that \textit{K. candel} is more tolerant to waterlogging. Nevertheless, when the contents of photosynthetic pigments increased due to prolonged waterlogging, the relative growth rate of both species did not increase, and even showed a significant decrease in \textit{B. gymnorrhiza} (Fig. 2). These results suggest that increases in photosynthetic pigments do not necessarily lead to more photosynthesis. Youssef and Saenger (1998) also found that under anoxic conditions (Eh $< -200$ mv) carbon assimilation rates of \textit{B. gymnorrhiza} seedlings were significantly lower but stomatal resistance was higher than under aerobic conditions (Eh $> 400$ mv). Therefore, increases in chlorophyll concentrations under anoxic conditions might not lead to an overall increase in photosynthesis as other factors such as stomatal resistance might reduce carbon assimilation rates.

Nitrate reductase catalyzes the first and rate-limiting step in the assimilation of nitrate (Beever and Hageman, 1969). Sung (1993) showed that imposition of waterlogging suppressed leaf nitrate reductase activity of soybean. In the present study, a significant increase in nitrate reductase activity in leaves of \textit{B. gymnorrhiza} and \textit{K. candel} waterlogged for 12 weeks (Fig. 5) suggests that these two species were able to tolerate waterlogging. Waterlogging for 12 weeks also resulted in nitrate reductase activity in \textit{K. candel} decreased significantly due to waterlogging, which was one of the reasons for a shift in biomass allocation from roots to shoots.

Lipid peroxidation was found to be more active in waterlogging-sensitive plants than in waterlogging-tolerant plants (Hunter et al., 1983). A dramatic increase in total superoxide dismutase activity of the resistant variety occurs under the anoxic environment but not in the sensitive ones (Monk et al., 1987). The activities of two anti-oxidation enzymes, peroxidase and superoxide dismutase, increased significantly in leaves of \textit{K. candel} when waterlogging period was longer than 8 weeks, while in \textit{B. gymnorrhiza}, only the peroxidase values show an increase and only little changes in superoxide dismutase activity (Fig. 7), indicating that \textit{K. candel} has stronger resistance to the oxidant damage due to waterlogging. This increase in superoxide dismutase activity was vital in protecting the plants against the oxidant damage from waterlogging stress (Bowler et al., 1992) and other stress (Takemura et al., 2000).

5. Conclusions

The present study clearly shows that \textit{K. candel} could stand longer waterlogging duration than \textit{B. gymnorrhiza}. This finding matches the geographi-
cal distribution of these two species in natural mangrove swamps in Hong Kong and China with *K. candele* more widely distributed in the foreshore and has some pioneering propensity while *B. gymnorrhiza* is more common in mid-tidal levels of a mangrove swamp. The result also implies that it would be more difficult to replant *B. gymnorrhiza* in low tide levels. Such information is extremely useful for mangrove conservation, in particular, how to select the appropriate site for mangrove replanting.

References


Tam, N.F.Y., Wong, Y.S., 2000. Hong Kong Mangroves. City University of Hong Kong Press, Hong Kong.


