

Photosynthetic responses of two mangrove species to salinity immersion and C3-CAM conversion

兩種紅樹林物種對鹽水淹沒的光合反應與 C3-CAM 轉化

C. W. Wang^{1*}, C. I. Chen², M. Y. Huang³, S. L. Wong¹, T. S. Liao⁴, J. H. Weng³,
and M. N. Chen⁵

王經文^{1*}、陳忠義²、黃盟元³、翁韶良¹、廖天賜⁴、翁仁憲³、陳明男⁵

^{1*}Endemic Species Research Institute, Nantou County, Taiwan

²Department of Forestry, National Pingtung University of Science and Technology,
Pingtung City, Taiwan

³Department of Life Sciences, Plant Ecophysiology, National Chung Hsing University,
Taichung City, Taiwan

⁴Department of Forestry, National Chung Hsing University, Taichung, Taiwan

⁵Department of Agriculture, Taoyuan City Government, Taoyuan City, Taiwan

¹ 行政院農業委員會特有生物研究保育中心 552 南投縣集集鎮民生東路 1 號

² 國立屏東科技大學森林學系 912 屏東縣內埔鄉學府路 1 號

³ 國立中興大學生命科學系 402 台中市南區國光路 250 號

⁴ 國立中興大學森林學系 402 台中市南區國光路 250 號

⁵ 桃園市政府 330206 桃園市桃園區縣府路 1 號

*Corresponding author: kilorsoul@tesri.gov.tw

* 通訊作者：kilorsoul@tesri.gov.tw

Abstract

Mangrove ecosystems are vulnerable to rising sea levels. When the sea level rises, plants are exposed to increased salinity as well as tidal submergence. In this study, *Kandelia obovata* Sheue, Liu & Yong and *Rhizophora stylosa* Griffith were subjected to different salinity (20‰ and 40‰) and immersion treatments to understand their photosynthesis responses to salinity immersion in mangroves and adaptability to different tidal elevations, gas exchange, and chlorophyll fluorescence parameters. *K. obovata* adapted to immersion stress and outperformed *R. stylosa* under these conditions, indicating that the colonization ability of *K. obovata* took place in the immersive conditions. Immersion and low CO₂ concentration stresses might enable *R. stylosa* to adapt to darkness for CO₂ absorption and exhibit leaf acidity in the early morning. Accordingly, *R. stylosa* exposed to immersion and low CO₂ concentration for one week could adapt to darkness and successfully absorb CO₂. This phenomenon was consistent with that observed in crassulacean acid metabolism (CAM)-type plants, which fix CO₂ at night and exhibit leaf acidity in the early morning. Our results demonstrate that *R. stylosa* displays distinct CAM characteristics under 40‰ of salinity and immersion stress.

Keywords: mangrove species, crassulacean acid metabolism, leaves pH, gas exchange, photoinhibition

摘要

紅樹林生態系統容易受到潮汐的影響，每當海平面上升時，植物會處在鹽度增加以及潮汐淹沒的環境中。為了解適應不同潮位高度的紅樹林鹽分淹水的光合作用，本試驗在不同鹽度（20‰ 和 40‰）與淹水處理下測量水筆仔 (*Kandelia obovata*, Liu & Yong) 與紅海欖 (*Rhizophora stylosa*, Griffith) 的氣體交換和葉綠素螢光參數。結果顯示水筆仔能適應淹水壓力並在這些條件下生理表現優於紅海欖。

而紅海欖處於淹水和低 CO₂ 濃度處理 1 週後，在夜晚裡能吸收 CO₂，並在清晨時葉片酸度較高，顯示淹水和低 CO₂ 濃度等多重逆境可能誘發紅海欖的景天酸代謝 (crassulacean acid metabolism) 的特徵，與景天酸代謝型植物一致。

關鍵詞：紅樹林樹種、景天酸代謝、葉片 pH、氣體交換、光抑制

Introduction

Mangroves are a diverse group of 70 species that grow in saline and tidal wetlands along tropical and subtropical coastlines (Lugo and Snedaker 1974; Odum *et al.* 1982, Hutchings and Saenger 1987; Alongi 2002). Mangrove habitats are typically characterized by high salinity, tidal influence, strong winds, high temperatures, and muddy anoxic soils (Kathiresan and Bingham 2001). Micro-tidal wetlands show strong seasonal soil salinity variation ranged from 0 ~ 1,709 mM of NaCl that are likely to increase in amplitude according to climate prediction models. This may affect the morphology and physiology of mangrove seedlings, and the growth and species composition of mangrove swamps as well (Bompy *et al.* 2014).

Globally, mangrove communities are experiencing changing flood depths and durations due to sea level rise (Woodroffe 1999). The halophytic nature of mangroves creates challenges for scientists to investigate the potential effects of altered hydroperiods. Freshwater inundation alone probably biases flood assessments because optimal growth and physiological activity are attained under saline conditions (Ball 1996, 2002). The effect of increased salinity on mangroves remains unknown. Salinity and fluctuations in flooded elevation are important factors that influence the distribution, niche patterns, and succession of mangrove species (Sabine *et al.* 2011). Mangroves generally have an optimal salinity range of 8–18‰ (Sabine *et al.* 2011) or an optimal seawater concentration range

of 5–75% (Krauss *et al.* 2008). In saline environments, *Rhizophora mangle* exhibits crassulacean acid metabolism (CAM) characteristics (Werner and Stelzer 1990), but mangrove species are widely considered to be C3 plants (Venkatesalu *et al.* 2008). Furthermore, photosynthesis and related physiological responses of mangrove species under high salinity are still not unveiled.

C3/CAM shifting requires the induction of certain stress factors. The major ecosystems with CAM plants submerged in aquatic sites have been described by Keeley (1996). The earliest evolution of CAM might have occurred in submerged plants. CAM is advantageous for surviving abiotic stress, rather than for dominance and high biomass production in poor oligotrophic sites (Lüttge 2004). Underwater photosynthesis in submerged aquatic plants is often limited by carbon dioxide availability and thus requires the development of CO₂-concentrating mechanisms (CCMs)

that increase carbon fixation of enzymes used by CAM and C4 carbon fixation through the induction of phosphoenolpyruvate carboxylase (PEPC), such as in submerged *Otteliaalismoides* (Huang *et al.* 2020) and *Hydrilla verticillata* (Holiday and Bowes 1980).

Certain plant species can switch from C3 metabolism to CAM either during ontogenetic development or under stress conditions, such as drought (Winter and Holtum 2007, Minardi *et al.* 2014, Holtum *et al.* 2017). In C3–CAM intermediate species, oxidative stress appears to be necessary for triggering a metabolic switch, although oxidative stress alone is insufficient to drive the C3–CAM shift (Lüttge 2004, Niewiadomska and Miszalski 2008). This view is supported by studies in which the C3–CAM plant *Mesembryanthemum crystallinum* was treated with O₃ and SO₂ (Hurst *et al.* 2004, Surówka *et al.* 2007). Osmond (1978) described four distinct stages of CAM in the diurnal cycle. In stage IV,

when the malate pool is exhausted at the end of the day, a decrease in the internal CO₂ level promotes stomatal opening, which enables the direct fixation of atmospheric CO₂ by Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). However, toward the end of Stage IV, RuBisCO activity declines, and PEPC is activated. Therefore, when the C3-CAM shift occurs, the main physiological indicators are an increase in leaf acidity in the early morning and a decrease in leaf acidity at sunset (Cushman and Bohnert 1999, Maxwell *et al.* 2002, Dodd *et al.* 2003).

The C3-CAM transition correlates with the transcriptional expression of PEPC genes (*Ppc1* and *GapC1*) and increased abscisic acid (ABA) during CAM induction (Cushman and Bohnert 1999). ABA accumulation has been proposed to be involved in CAM induction (McElwain and Bohnert 1992). The switch to CAM is determined by age-dependent development owing to its

genetic program (Cushman and Bohnert 1999). Adversity increases physiological leaf age and enhances the rate of ABA catabolism (Cornish and Zeevaart, 1984).

The objectives of our study were to (1) develop an easy, rapid, and noninvasive method for instantaneous assessment of dynamic photochemical efficiency based on photosynthesis and chlorophyll fluorescence parameters, and (2) measure the pH of leaves with minimal destructive sampling. These parameters reflect a physiological response that could be used to evaluate tidal stress in *Kandelia obovata* and *Rhizophora stylosa*. The obtained data can serve as indicators of plant survival strategies. The hypothesis of our study was that *K. obovata* could adapt to tidal stress, whereas *R. stylosa* would be adversely affected by tidal stress.

Materials and methods

Plant materials and treatments: The

seedlings of *Kandelia obovata* Sheue, Liu & Yong and *Rhizophora stylosa* Griffith in the Tainan Shuangchun Estuary (23°17'38.8"N, 120°06'42.3"E) were collected in 2015 and planted in the Endemic Species Research Institute (23°49'43.0"N, 120°48'04.7"E). The cultivation medium was estuarian sea sand and the seedlings were cultivated in round plastic buckets (diameter, 20 cm; height, 20 cm). Salinity treatment was initiated when the seedlings were two years old, seedling height was 50-60 cm, and ground diameter was 3-4 cm in July, 2017. We prepared two kinds of salinity solutions (20‰ and 40‰) with 20 pots (five for dark period measurements and 15 for standard measurements) of seedlings for each species. The treatment was performed weekly to ensure that salinity was maintained at 20‰ and 40‰ until six months. Moreover, we measured photosynthesis and chlorophyll fluorescence parameters described in detail below.

Whole plant flooding treatment:

Flooding treatments were performed for seven days from April to June, 2018. Two year-old plants were exposed to intermittent flooding for four hours each morning (06:00–8:00 h) and afternoon (16:00–18:00 h) to simulate tidal influences. The flooding treatment container was a plastic bucket with a diameter of 56 cm and height of 75 cm. The salinity of the flooding solution remained constant throughout the treatment. The control group received no flooding treatments. During the flooding treatment, we studied the effects of reducing CO₂ supply and salt concentration of the leaves.

Low-CO₂-concentration treatment

of leaves: From April to June, 2018, soda-lime was used as a CO₂ absorber or absorbent in the low-CO₂ treatment (0–10 ppm) of leaves for seven days each morning (06:00–8:00 h) and afternoon (16:00–18:00 h) during this intermittent treatment period, the mangrove

leaves were exposed to low $[\text{CO}_2]$ for four hours to decrease CO_2 supply during tidal flooding and the influence of no-salt on leaf blades during flooding. Control plants were not flooded, and CO_2 supply to these plants was not reduced. In addition, the no-salt leaves were not affected by salt during flooding.

Photosynthesis and chlorophyll fluorescence parameters with a fixed light source:

We divided *K. obovata* and *R. stylosa* seedlings into four groups according to the treatment: (1) 20‰ of salt without immersion, (2) 20‰ of salt with immersion, (3) 40‰ of salt without immersion, and (4) 40‰ of salt with immersion, with five replicates per treatment and species. From April to June, 2018, the seedlings were measured using a gas exchange and fluorescence photosynthesis analyzer (GFS-3000FL; Walz, Effeltrich, Germany). After flooding, photosynthetic parameters were measured at 10:00 h

each day.

During the experiment, the gas flow rate was set to $750 \mu\text{mol s}^{-1}$, gas-mixer speed to the 7-level, assimilator temperature to 25°C , and relative humidity to 75%, and the photosynthetic photon flux density (PPFD) was adjusted to $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. Photosynthesis, gas exchange, and chlorophyll fluorescence parameters were measured for 90 min of irradiation and then during dark adaptation for 30 min. We recorded one data point every minute for the first 30 min and one data point every 2 min for the final 90 min to measure the photosynthesis, gas-exchange, and chlorophyll fluorescence parameters, and then calculated the following two parameters (Demmig-Adams and Adams 1996, Johnson and Ruban 2011):

$F_v/F_m = (F_m - F_o)/F_m$, where F_v , F_m , and F_o are values of the minimal (F_o), and maximal ChlF (F_m) of dark-adapted samples were determined using modulated irradiation of a weak light-emitting di-

ode beam (measuring light) and saturating pulse, respectively. Photoinhibition of dark recovery = $(F_v/F_m - F_{v_T}/F_{m_T}) / F_v/F_m$, where F_{v_T} and F_{m_T} are values measured at 2 and 30 min, respectively, after dark recovery (Wang et al. 2022) measurements.

CO₂ absorption measurement in dark period: From April to June, 2018, five seedlings of *K. obovata* and *R. stylosa* were subjected to (1) immersion, (2) low [CO₂], and (3) no immersion for the measurement of CO₂ absorption in the dark period under 40‰ salinity treatment. A gas-exchange analyzer was used after the leaves submerged in water and treated with low [CO₂] daily. The [CO₂] uptake and gas-exchange parameters were measured in the dark period at 18:00 h every day and recorded every 2 min with a total of 250 records.

Leaf pH measurement: The leaf pH of *K. obovata* and *R. stylosa* seedlings sub-

jected to (1) immersion, (2) low [CO₂], and (3) no immersion were also measured under 40‰ salinity treatment from April to June, 2018. Measurements were performed daily at 06:00 h and 18:00 h. The leaves were ground into powder and mixed with deionized water (10 mL of water per 1 g of sample). After mixing, the samples were vortexed for 5 min. The pH of the mixed solution was measured using a portable pH meter (HQ11d, Hach, USA).

Statistical analysis: Statistical analyses were performed using PASW Statistics 18 and SigmaPlot 10.0 software. Gas exchange and chlorophyll fluorescence were analyzed using a single-factor analysis of variance to check for significant differences between the two species. Multiple comparisons were performed using the least significant difference (LSD) method. A Pearson correlation coefficient analysis between gas exchange and chlorophyll fluorescence parameters

was also performed.

Results

CO₂ absorption in the dark period and leaf pH

R. stylosa exhibited a night dark-adapted state for CO₂ uptake within 250 min in both low [CO₂] and immersion treatments (Fig. 1). The maximum CO₂ absorption rates under night dark measured for *R. stylosa* were 9.27 μmol CO₂ m⁻² s⁻¹, which was similar to the rate of CO₂ fixed at night for photosynthesis in CAM plants, as well as the rate in the fourth stage of CAM. Moreover, the pH of the *R. stylosa* leaves measured at 06:00 h was significantly ($P < 0.05$) lower than that at 18:00 h under a dark-adapted state for CO₂ uptake after four hours of treatment with CO₂ supply reduction and daily immersion for one week (Fig. 2). Therefore, *R. stylosa* is characteristically similar to those of CAM plants.

Photosynthesis rate and fluorescence re-

sponse of photoinduction

In this experiment, 800 μmol photon m⁻² s⁻¹ PPFD was used to induce the photosynthesis rate (P_N) in *K. obovata* and *R. stylosa* and explore the photosynthetic physiological response. Due to the delayed reaction of the P_N , photosynthesis and stomatal conductance (gs) were examined over three time periods: 30 min, 60 min, and 90 min. Figure 3 shows that the P_N and gs values of *K. obovata* and *R. stylosa* seedlings during the 30 min period were relatively lower than both 60 min and 90 min. Regardless of the low [CO₂] or immersion treatment, the P_N and gs of *K. obovata* significantly increased after 60 and 90 min of irradiation. The low [CO₂] and immersion treatment seedlings under 40 ‰ salinity showed significantly higher rates compared to no immersion seedlings ($P < 0.05$). However, in *R. stylosa*, the P_N and gs with no immersion treatment increased significantly after 90 min of irradiation, and the seedlings under 20‰ salinity treatment

exhibited significantly higher values than those under the 40‰ salinity treatment ($P < 0.05$). Meanwhile, low $[CO_2]$ and immersion seedlings under 40 ‰ salinity still exhibited low values after 90 min of irradiation, and the values were significantly lower than those of the no immersion seedlings ($P < 0.05$), indicating that low $[CO_2]$ and immersion at high salinity had negative effects on *R. stylosa*.

As shown in Fig. 4A, after 40‰ salinity treatment, *K. obovata* seedlings were negatively affected by high salinity. Before irradiation, Fv/Fm values of *K. obovata* seedlings under no immersion, low $[CO_2]$, and immersion treatment were 0.76 ± 0.06 , 0.80 ± 0.01 , and 0.78 ± 0.05 , respectively. After $800 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ irradiation for 90 min followed by acclimation for 30 min in the dark period, the Fv/Fm values of *K. obovata* seedlings under no immersion, low $[CO_2]$, and immersion treatment decreased to 0.61 ± 0.09 , 0.69 ± 0.03 , and 0.69 ± 0.04 , respectively (Fig. 4A), but the photoin-

hibition ratio was approximately 20% (Fig. 4C). These results demonstrate that decline rate of *K. obovata* was larger in no immersion seedlings compared to low $[CO_2]$ and immersion treated seedlings. The photoinhibition ratio of no immersion *K. obovata* seedlings treated with 20 ‰ salinity was approximately 10%, suggesting that the decline rate of *K. obovata* seedlings in 20 ‰ salinity treatment was greater than that in 40 ‰ salinity treatment.

Fv/Fm values of 40‰ salinity-treated *R. stylosa* seedlings exposed to no immersion, low $[CO_2]$, and immersion before irradiation were 0.73 ± 0.009 , 0.69 ± 0.06 , and 0.74 ± 0.02 , respectively (Fig. 4B), demonstrating that *R. stylosa* was affected by stress in a high salinity environment. When the irradiation was adjusted in the dark period, all Fv/Fm values of *R. stylosa* seedlings exposed to no immersion, low $[CO_2]$, and immersion dropped to 0.60 ± 0.03 , 0.49 ± 0.055 , and 0.60 ± 0.03 , respectively (Fig. 4B), but

the photoinhibition ratio was approximately 20% (Fig. 4D). After 20 ‰ salinity treatment, the decrease in F_v/F_m after irradiation was small, whereas the negative effect of flooding was large. These results show that the long-term exposure of *K. obovata* and *R. stylosa* to high-salinity environments had a negative impact. In addition, significant photoinhibition occurred when the irradiation time exceeded 90 min under $800 \mu\text{mol photon m}^{-2} \text{s}^{-1}$.

Discussion

C3/CAM shifting is induced by certain stress factors, such as CO_2 concentration reduction due to inundation (Huang *et al.* 2020), drought (Holtum *et al.* 2017), salinity (Gawronska *et al.* 2013), and high light (Hurst *et al.* 2004). Oxidative stress appears to be necessary to induce adaptation to CO_2 uptake in the dark in C3/CAM shifting species; however, stress alone is insufficient to drive C3/CAM shifting, among which need to

be induced by other stresses, such as salinity (Lüttge 2004, Niewiadomska and Miszalski 2008).

In our study, dark-adaptation in *R. stylosa* induced CO_2 uptake after four-hour treatment of flooding and low $[\text{CO}_2]$ treatment daily for one week (Fig. 4B). Furthermore, CO_2 absorption under night-dark adaptation in *R. stylosa* was similar to the rates at which CO_2 was fixed at night for photosynthesis in CAM plants at Stage IV (Osmond 1978). When malic acid is depleted at the day-end (Osmond 1978), a decrease in internal CO_2 levels promotes stomatal opening, which enables RuBisCO to fix atmospheric CO_2 directly. Gawronska *et al.* (2013) reported that *M. crystallinum* must be treated under saline conditions to generate factors that induce CAM traits. When *R. stylosa* was exposed to 40‰ salinity treatment for four hours at low $[\text{CO}_2]$ and flood for a period of one week, the plant experienced multiple stresses from salinity, oxidation, and excessive light, which

enabled it to absorb CO₂ in the dark period. The pH of the leaves measured at 06:00 h was significantly lower than that at 18:00 h. Gawronska *et al.* (2013) treated *M. crystallinum* with 0.4 M NaCl for 12 days and found that transformed photosynthetic system from the C3 system to a CAM system.

Delayed activation of CAM plants was associated with levels of RuBisCO activase protein, which reached a maximum at midday, and may account for this pattern of RuBisCO activation, such as under drought stress or incubation of leaves overnight in an oxygen-free atmosphere, releasing RuBisCO from inhibition early in the light period (Maxwell *et al.* 2002). This is comparable to *R. stylosa*, where P_N and g_s remained near 0 under low CO₂ and immersion for 40‰ salinity treatment (Figs. 3B and D).

The photoinhibition shown in Fig. 4 suggests that photosynthesis was inhibited by the increased photoinhibition under high salt concentrations. P_N and

g_s of *R. stylosa* were almost completely inhibited under low CO₂ and immersion for 40‰ salinity treatment. Compared with the experimental results of Huang *et al.* (2020), in our study, Fv/Fm decreased when plants switched from C3 to CAM, suggesting that environmental stress affects the results of seedlings.

References

- Alongi DM (2002) Present state and future of the world's mangrove forests. *Environ Conserv* 29(3): 331–349.
- Ball MC (1996) Comparative ecophysiology of mangrove forest and tropical lowland moist forest. – In: Mulkey S.S., Chazdon R.L., Smith A.P. (ed.): *Tropical forest plant ecophysiology*. Pp. 461–296. Chapman and Hall, New York.
- Ball MC (2002) Interactive effects of salinity and irradiance on growth: implications for mangrove forest

- structure along salinity gradients. *Trees* 16: 126–139.
- Bompy F, Lequeue G., Imbert D, Dulorme M (2014) Increasing fluctuations of soil salinity affect seedling growth performances and physiology in three Neotropical mangrove species. *Plant Soil* 380(1–2): 399–413.
- Cornish K, Zeevaart JA (1984) Abscisic Acid Metabolism in Relation to Water Stress and Leaf Age in *Xanthium strumarium*. *Plant Physiol* 76(4):1029-1035.
- Cushman JC, Bohnert HJ. (1999) Crassulacean acid metabolism: molecular genetics. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 305-332.
- Demmig-Adams B, Adams III WW (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci* 1:21–26.
- Dodd AN, Griffiths H, Taybi T, Cushman JC, Borland AM (2003) Integrating diel starch metabolism with the circadian and environmental regulation of Crassulacean acid metabolism in *Mesembryanthemum crystallinum*. *Planta* 216:789–797.
- Gawronska K, Romanowska E, Miszalski Z (2013) Limitation of C3-CAM shift in the common ice plant under high irradiance. – *J Plant Physiol.* 170: 129-135.
- Holaday A. S., Bowes G. (1980) C4 Acid Metabolism and Dark CO₂ Fixation in a Submersed Aquatic Macrophyte (*Hydrilla verticillata*). *Plant Physiol.* 65, 331-335.
- Holtum JAM, Hancock LP, Edwards EJ, Winter K (2017) Facultative CAM photosynthesis (crassulacean acid metabolism) in four species of *Calandrinia*, ephemeral succulents of arid Australia. *Photosynth Res* 134:17–25.
- Huang W, Jin Q, Yin L, Li W (2020)

- Responses of CO₂-concentrating mechanisms and photosynthetic characteristics in aquatic plant *Ottelia alismoides* following cadmium stress under low CO₂. *Ecotoxicology and Environmental Safety* 202: 1-9.
- Hutchings P, Saenger P (1987) Ecology of mangroves. University of Queensland Press, Brisbane. Pp. 689–701.
- Hurst AC, Grams TEE, Ratajczak R (2004) Effects of salinity, high irradiance, ozone, and ethylene on mode of photosynthesis, oxidative stress and oxidative damage in the C3/CAM intermediate plant *Mesembryanthemum crystallinum* L. – *Plant Cell Environ.* 27: 187-197.
- Johnson MP, Ruban AV (2011) Restoration of rapidly reversible photoprotective energy dissipation in the absence of PsbS protein by enhanced Δ pH. *J Biol Chem* 286:19973–19981.
- Kathiresan K, Bingham BL (2001) Biology of mangroves and mangrove ecosystems. *Adv Mar Biol* 40: 81–251.
- Keeley JE (1996) Aquatic CAM photosynthesis. In: Winter K, Smith JAC, eds. *Crassulacean acid metabolism. Biochemistry, ecophysiology and evolution. Ecological Studies vol 114.* Berlin, Heidelberg, New York: Springer-Verlag, 281-295.
- Krauss KW, Lovelock CE, McKee KL, López-Hoffman L, Ewe SML, Sousa WP (2008) Environmental drivers in mangrove establishment and early development: A review. *Aquatic Bot* 89: 105–127.
- Lugo AE, Snedaker SC (1992) The ecology of mangroves. *Ann Rev Ecol Syst* 5: 39–64, 1974.
- Lüttge U (2004) Ecophysiology of crassulacean acid metabolism (CAM). – *Ann Bot.* 93: 629-652.

- Maxwell K, Griffiths H, Helliker B, Roberts A, Haslam RP, Girnus J, Robe WE, Borland AM (2002) Regulation of Rubisco activity in crassulacean acid metabolism plants: better late than never. – *Funct Plant Biol.* 29: 689-696.
- McElwain E.F., Bohnert H.J., Thomas J.C., (1992) Light mediates the induction of phosphoenolpyruvate carboxylase by NaCl and abscisic acid in *Mesembryanthemum crystallinum* L., *Plant Physiol.* 86: 180–187.
- Minardi BD, Voytena APL, Santos M, Randi ÁM (2014) Water stress and abscisic acid treatments induce the CAM pathway in the epiphytic fern *Vittaria lineata* (L.) Smith. *Photosynthetica* 52: 404–412.
- Niewiadomska E, Miszalski Z (2008) Partial characterization and expression of leaf catalase in the CAM-inducible halophyte *Mesembryanthemum crystallinum* L. – *Plant Physiol Biochem.* 46: 421-427.
- Odum WE, McIvor CC, Smith III TJ (1982) The ecology of the mangroves of south Florida: A community profile. US Fish and Wildlife Service, Office of Biological Services, Washington, DC FWS/OBS-81/24.
- Osmond CB (1978) Crassulacean acid metabolism: a curiosity in context. – *Annu Rev Plant Physiol.* 29: 379-414, 1978.
- Sabine CL, Speera H, Adamsa JB, Rajkaran A, Bailey D (2011) The response of the red mangrove *Rhizophora mucronata* Lam. to salinity and inundation in South Africa. *Aquatic Bot* 95: 71–76.
- Surówka E, Karolewski P, Niewiadomska E, Libik M, Miszalski Z (2007) Antioxidative response of *Mesembryanthemum crystallinum* plants to exogenous SO₂ applica-

- tion. – Plant Sci. 172: 76-84.
- Venkatesalu V, Senthilkumar A, Chandrasekaran M, Kannathasan K (2008) Screening of certain mangroves for photosynthetic carbon metabolic pathway. *Photosynthetica* 46(4): 622-626.
- Wang CW, Wong SL, Liao TS, Weng JH, Chen MN, Huang MY, Chen CI (2022) Photosynthesis in response to salinity and submergence in two Rhizophoraceae mangroves adapted to different tidal elevations. – *Tree Physiol.* 42: 1016-1028.
- Winter K, Holtum JAM (2007) Environment or development? Lifetime net CO₂ exchange and control of the expression of crassulacean acid metabolism in *Mesembryanthemum crystallinum*. *Plant Physiol* 143:98–107.
- Werner A, Stelzer R (1990) Physiological responses of the mangrove *Rhizophora mangle* grown in the absence and presence of NaCl. *Plant Cell Environ* 13:243–255.
- Woodroffe CD (1999) Response of mangrove shorelines to sea-level change. *Tropics* 8: 159–177.

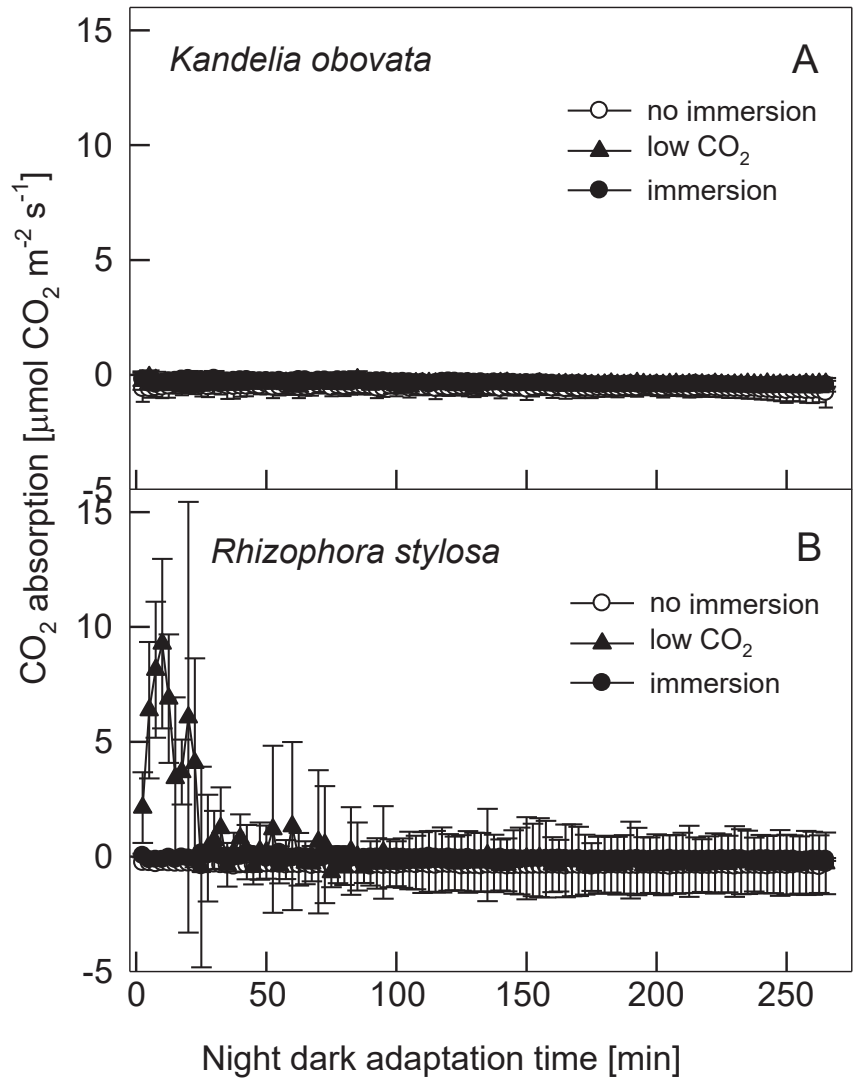


Fig.1. CO₂ absorption under night-dark adaptation in *Kandelia obovata* (A) and *Rhizophora stylosa*. *K. obovata* (B) subjected to no immersion, low [CO₂], and immersion treatments at 40‰ salinity. The figure shows the changes in CO₂ absorption under night dark adaptation. Error bar = standard error (SE); n = 5.

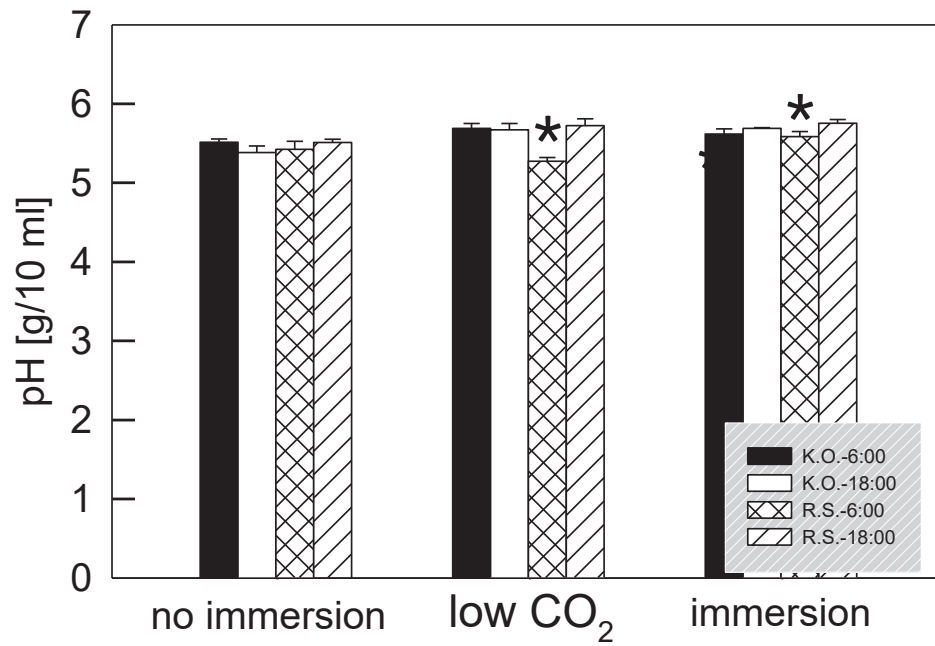


Fig.2. *Kandelia obovata* and *Rhizophora stylosa* subjected to 40‰ salinity treatment with no immersion, low [CO₂], and immersion provided with daily supply of CO₂ for four hours. Changes in leaf pH (1 g/10 mL) were measured at 06:00 h and 18:00 h after one week. Error bar = SE; n = 5. *P < 0.05.

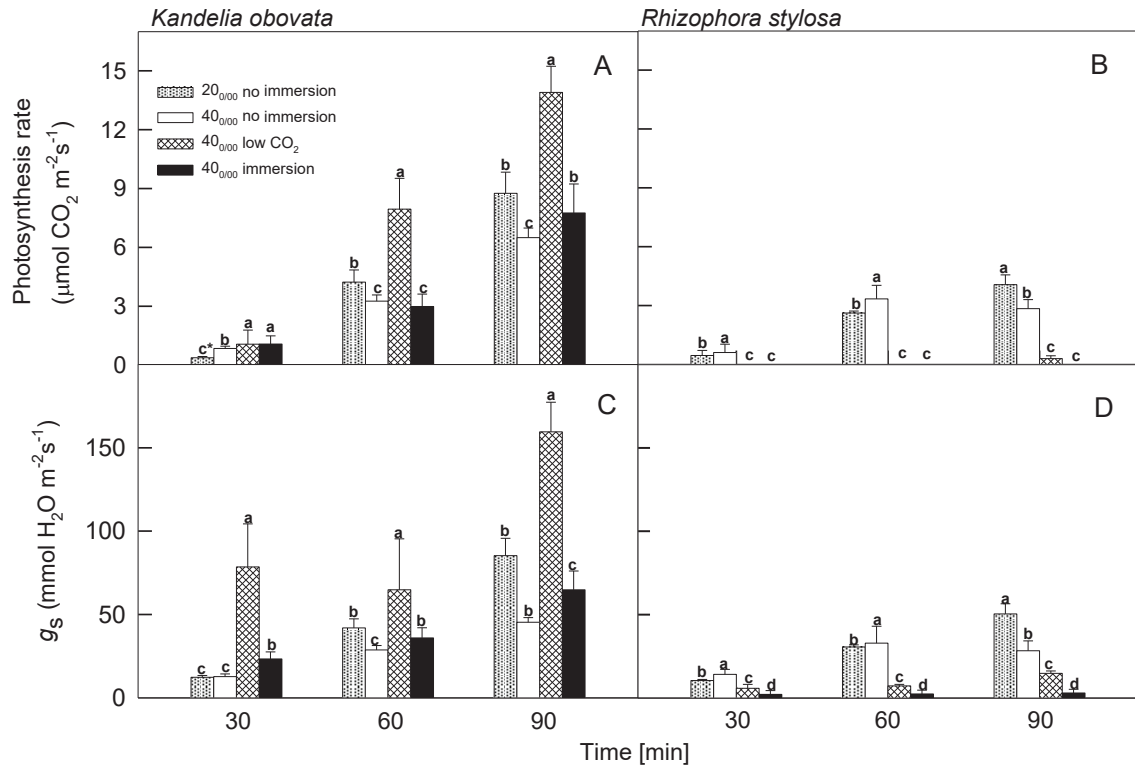


Fig.3. Net photosynthesis rate (PN) and stomatal conductance (g_s) of leaves of *Kandelia obovata* (A, C) and *Rhizophora stylosa* (B, D) seedlings. Plants were subjected to no immersion at 20‰ and 40‰ salinity, low [CO₂] at 40‰ salinity, and immersion at 40‰ salinity treatments under 800 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ PPFD after 30, 60, and 90 min of irradiation. Error bar = SE; n = 5. * Different letters indicate significant differences in the LSD analyses between treatments at different salinity and immersion at the same time (P < 0.05).

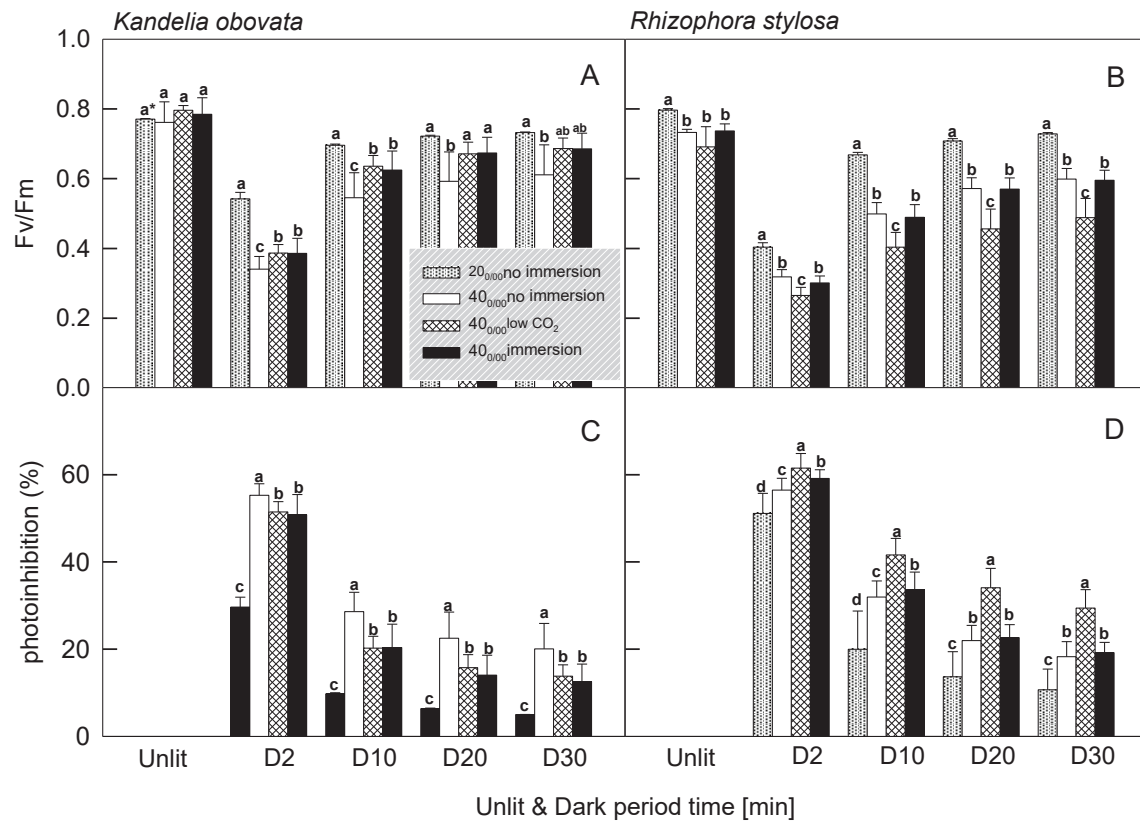


Fig. 4. Fv/Fm and photoinhibition (%) of *Kandelia obovata* (panels A and C) and *Rhizophora stylosa* (panels B and D) were measured after 90 min of 800 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ irradiation and dark period exposure for 2 min (D2), 10 min (D10), 20 min (D20), and 30 min (D30) under no immersion at 20‰ and 40‰ salinity, low [CO₂] at 40‰ salinity, and immersion at 40‰ salinity treatments. Error bar = SE; n = 5. *Different letters indicate significant differences in the LSD analyses between treatments at different salinity and immersion at the same time (P < 0.05).