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The photosynthesis and chlorophyll *a* fluorescence in seedlings of *Kandelia candel* (L.) Druce grown under different nitrogen and NaCl controls

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Abstract

Kandelia candel (L.) Druce is the dominant mangrove species on the west coast of northern Taiwan. We have measured the net photosynthetic rate (P_N) and chlorophyll (Chl) *a* fluorescence of seedlings grown at combinations of two nitrogen (0.01 and 0.1 mM) and two NaCl (250 and 430 mM NaCl) controls. With the same nitrogen level, seedlings grown at higher salinity (HS) had a significantly lower P_N and stomatal conductance (g_s) than those at lower salinity (LS). An increase in nitrogen availability significantly elevated P_N and g_s of the LS-grown seedlings. Compared to dark adapted leaves, the maximum quantum yield of photosystem 2 (PS2) (F_v/F_m) of leaves exposed to PFDs of 1200 and 1600 μmol m^{-2} s^{-1} for 2 h was significantly reduced. The degree of F_v/F_m reduction differed among leaves of the four types of treated plants. Chl fluorescence quenching analysis revealed differences among the examined plants in coefficients of non-photochemical and photochemical quenching.

Additional key words: mangrove; net photosynthetic rate; photoinhibition; photoprotection; salinity; stomatal conductance.

Introduction

The growth of mangrove in intertidal sediments is often limited by a low nitrogen availability and high salinity (Lugo and Snedaker 1974, Tomlinson 1986). The low N

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Abbreviations: Chl - chlorophyll; F_0'- minimal fluorescence in the light-adapted state; F_m - maximum fluorescence in the dark-adapted state; F_m' - maximum fluorescence in the light; F_t - steady-state fluorescence in the light-adapted state; F_v - variable chlorophyll fluorescence in the dark-adapted state; F_v' - variable fluorescence in light; HN - high nitrogen treated plants; HS - high NaCl treated plants; LN - low nitrogen treated plants; LS - low NaCl treated plants; P_N - net photosynthetic rate; PFD - photon flux density; PS - photosystem; Q_A - primary electron acceptor of photosystem 2; q_N - non-photochemical quenching; q_P - photochemical quenching.

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availability results in reductions of the carbon assimilation rate, that can be partially attributed to a lesser investment of N into the photosynthetic machinery (Field and Mooney 1986). In addition to this, the high salinity may cause high leaf water deficits and low g_s (Ball 1988). Low P_N and low g_s typical of mangroves mean that sun leaves receive an excess of excitation energy (Björkman *et al.* 1988, Cheeseman 1994). The phenomenon of photoinhibition occurs when the leaves are exposed to irradiances exceeding what can be utilized in photosynthesis (Powles 1984), and PS2 is considered the primary site of photoinhibition (Barber and Andersson 1992).

Chl fluorescence of PS2 reveals the response of photosynthesis to environmental stresses (Lichtenthaler and Rinderle 1988, Baker 1991). In particular, the maximum quantum yield of PS2 (F_v/F_m), the efficiency of excitation capture by open PS2 (F_v'/F_m'), and the photochemical and non-photochemical quenching coefficients (q_P and q_N - Schreiber *et al.* 1994) provide important information of photosynthetic activity. The linear relationship between quantum yield and F_v/F_m (Adams *et al.* 1990) suggests that F_v/F_m can monitor the photosynthetic carbon assimilation (Björkman 1987). A reduction in F_v/F_m of dark-adapted leaves indicates photoinhibition of PS2. Additionally, the irradiance responses of PS2 photochemistry and the relative radiant energy-saturated rates among different species show strong resemblances to the corresponding responses and radiant energy-saturated rates of CO_2 uptake (Björkman and Demmig-Adams 1994). Hence, the application of Chl fluorescence technique is a rapid and convenient method for non-destructive estimates of photosynthetic performance.

Kandelia candel is the dominant mangrove species on the west coast of northern Taiwan (Liu 1982). In a previous experiment, we found that nitrogen and NaCl affect the growth of this plant species (Kao *et al.*, unpublished). Thus, although *K. candel* is able to colonize saline habitat, but high salinity and nutrient availability levels limit its growth (Kao and Chang 1998), nobody has studied the mechanism(s) of these effects on growth of the plant yet. In the present study, we measured P_N and Chl fluorescence of seedlings of *K. candel* grown at combinations of two nitrogen availability and two salinity regimes with the aim to understand their effects on its photosynthesis.

Materials and methods

Mature propagules of *K. candel* were collected from the Chu-wei Mangrove Nature Reserve (25°9'N, 121°26'E) in northern Taiwan during April 1998. The salinity of this area varies from 5 to 25 ‰ (Liu 1996). The propagules of similar length (20-25 cm) were grown in 0.1 m diameter plastic pots filled with sand at a density of one propagule per pot inside a glasshouse of the Academia Sinica, Taipei in natural daylight. The pots were put into a modified Hoagland's solution (Haines and Dunn 1976) containing 85 mM NaCl. Nitrogen and NaCl treatments began when the propagules produced roots and the cotyledons were fully expanded. The experimental design was a completely randomized split-plot, containing one species, 0.01 (LN) and 0.1 mM (HN) NH_4NO_3 fertilization, 250 (LS) and 430 mM (HS) NaCl treatments, and six replicate seedlings. The culture solutions were not aerated, but they were renewed every 2 weeks. The measurements were taken on plants which had been subjected to treatments for two months.

The most recently developed, fully expanded leaf was enclosed in a leaf cuvette (2×3 cm) of an steady-state open gas exchange system (*Li-Cor 6400, Li-Cor*, Lincoln, USA) and its P_N and g_s were measured. The conditions within the cuvette were controlled at ambient CO_2 concentration of 360 cm^3 m^{-3}, air temperature of 35 °C, and water vapour concentration difference between leaf and air of 2 mmol mol^{-1}.

After an exposure to sunlight for 2 h, leaves were dark adapted for 40 min at 30 °C before the measurement of F_v/F_m started. The F_v/F_m values were determined using a pulse amplitude modulated fluorometer (*PAM 2000, Walz*, Effeltrich, Germany). The photosynthetic photon flux density (PFD) was measured with a hand-held photometer (*Li-190, Li-Cor*, Lincoln, USA). To measure the Chl fluorescence-PFD response, a 2×3 cm piece of leaf was held horizontally in a temperature controlled cuvette (see above) under a fiber illuminator (*FL-440, Walz*, Germany). After the leaf was exposed to a desired PFD for 10 min, the Chl *a* fluorescence of PS2 was measured using the same fluorometer. During measurements, the leaf temperature was kept at 35 °C. The effective quantum yield of PS2 $[(F_m' - F_t)/F_m']$, the coefficients of photochemical quenching, $q_P = (F_m' - F_t)/(F_m' - F_0')$ and non-photochemical quenching, $q_N = (F_m - F_m')/(F_m - F_0')$ were computed (Schreiber *et al.* 1986), where F_m' is the maximal fluorescence, F_0' is the minimal fluorescence, F_v' is the difference between F_m' and F_0', and F_t is the steady-state fluorescence in the light-adapted state.

The total N content of leaf samples was analyzed with an elemental analyzer (*NA 1500, Fisons*, Italy). The Na content was determined from 6 M HCl extracts prepared from the ground leaf material (Lambert 1976), then subsequently analyzed with a flame photometer (model *410, Corning*, England). All statistical tests were performed using the computer software *SYSTAT* (*Statistical Solutions Limited*, Cork, Ireland). Significant levels are reported as $p<0.05$.

Results

The nitrogen and sodium contents of leaves were affected by different N and NaCl treatments: The HN-treated plants had significantly higher N contents than the LN-treated ones (Table 1). The HS-grown plants also had a significantly higher Na content than the LS ones (Table 1). Plants of the same N availability but different salinity treatments were not significantly different in their N contents.

The photosynthetic gas exchange: The HS seedlings had lower P_N and g_s than the LS ones at the same N treatment (Table 1). At LS, LN treatment significantly reduced P_N and g_s of the seedlings ($p<0.05$). At HS, these parameters were also lower in LN- than in HN-treated ones, but the reduction effect was not significant ($p>0.05$).

Table 1. Means and standard errors (S.E.) ($n = 6$) of nitrogen and Na contents [g kg^{-1}(d.m.)], net photosynthetic rate, P_N [μmol m^{-2} s^{-1}], and stomatal conductance, g_s [mmol m^{-2} s^{-1}] of *K. candel* grown at different nitrogen (HN = 0.1 and LN = 0.01 mM) and NaCl (HS = 430 and LS = 250 mM) treatments. Means within rows followed by different superscripts are different at $p = 0.05$ (Tukey's test).

Treatment	LSHN	LSLN	HSHN	HSLN
N	26 ± 1^a	16 ± 1^b	23 ± 1^a	19 ± 1^b
Na	28 ± 1^a	25 ± 2^a	37 ± 1^b	38 ± 1^b
P_N	9.9 ± 0.5^a	6.8 ± 0.7^b	6.3 ± 0.4^c	4.9 ± 0.2^c
g_s	143 ± 12^a	90 ± 16^b	77 ± 8^b	58 ± 5^b

F_v/F_m: No significant difference was found in F_v/F_m either between seedlings of different N treatments or between those of different NaCl treatments when the leaves were kept in darkness (Fig. 1). However, the exposure of the leaves to sunlight (PFD of 1200 or 1600 μmol m^{-2} s^{-1}) for 2 h significantly reduced the F_v/F_m value of leaves of all treatments indicating a decline in photosynthetic quantum conversion possibly related to photoinhibition. In general, the reduction in F_v/F_m was greater in the leaves exposed to a PFD of 1600 μmol m^{-2} s^{-1} than in those exposed to a PFD of 1200 μmol m^{-2} s^{-1}. Nevertheless, the F_v/F_m ratio was significantly higher in LS than HS plants. Under the same values of salinity treatment and PFD, generally the leaves of LN-plants had lower F_v/F_m values than those of HN-plants. In the consequence, among all the treated seedlings, leaves of HSLN grown plants had the lowest F_v/F_m, when exposed to PFD of 1600 μmol m^{-2} s^{-1}.

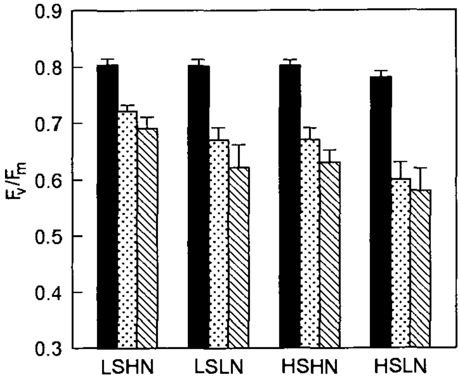

Fig. 1. Effects of photosynthetic photon flux density (PFD) on the ratio of F_v/F_m of *K. candel* grown at different nitrogen (HN = 0.1 and LN = 0.01 mM) and NaCl treatments (HS = 430 and LS = 250 mM). Leaves were dark adapted for 40 min at 30 °C after being exposed to a PFD of 0 (*first columns*), 1200 (*second columns*), or 1600 (*third columns*) μmol m^{-2} s^{-1} for 2 h. Means from 6 different plants, standard errors are indicated by bars.

Chl fluorescence-PFD response: Among the four types of treated plants, LSHN-plants had the highest, HSHN- and LSLN-plants intermediate, and HSLN-plants the lowest effective quantum yield (Fig. 2*A*) compared at the same PFD. At a PFD<400 μmol m^{-2} s^{-1}, a significant difference in q_P among the four treatments was not observed indicating that at lower PFDs, the leaves of all four types had a similar proportion of reduced to oxidised Q_A (the primary electron acceptor of PS2 = $1 - q_P$). In contrast, as PFD increased, the values of q_P were significantly higher in the leaves of LSHN-plants than in the leaves of LSLN-plants. The q_P values of HS-plants were also higher in the leaves grown at HN than at LN, however, the differences were not as steep as those in LS-plants. The q_N value in the leaves of LN grown plants rose quicker and was bigger than that of HN-plants at PFD<1100 μmol m^{-2} s^{-1} (Fig. 2*C*). Comparing plants that received the same N-treatment, significantly higher q_N values were found in HS- than LS-plants, however, no difference was found between HSHN and LSHN when PFD exceeded 1100 μmol m^{-2} s^{-1}.

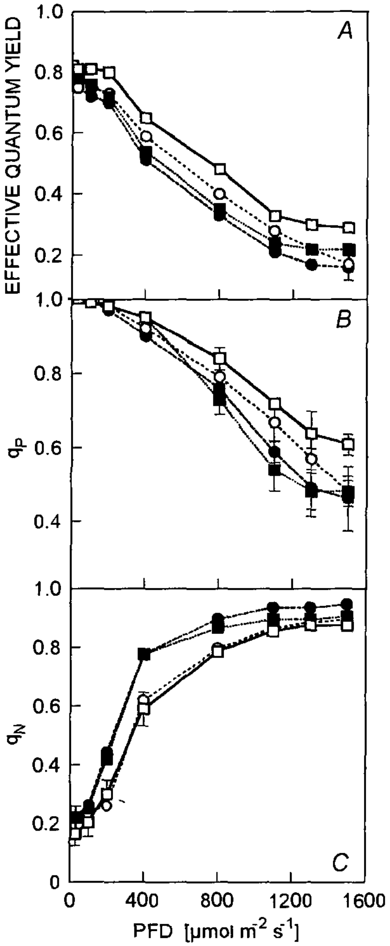

Fig. 2. Response of effective quantum yield of photosystem 2 (*A*), coefficient of photochemical chlorophyll (Chl) fluorescence quenching (q_P) (*B*), and coefficient of non-photochemical Chl fluorescence quenching (q_N) (*C*) to irradiance (PFD) of *K. candel* grown at two nitrogen availabilities, HN (*open*) = 0.1 and LN (*filled*) = 0.01 mM, and two NaCl treatments, HS (*circles*) = 430 and LS (*squares*) = 250 mM. Means from 6 different plants; standard errors are indicated by bars, if larger than symbols.

Discussion

P_N of leaves of *K. candel* grown at LS was limited by nitrogen availability, HN-treatment increased their P_N (Table 1). However, at HS, the P_N was more limited by salinity than by N-availability. Consequently, increase in N-availability had a limited effect on the enhancment of P_N of *K. candel* grown HS.

The effect of salinity on Chl fluorescence differs among plants species studied. For example, Brugnoli and Björkman (1992) reported that F_v/F_m in cotton was not affected by salinity. However, Björkman *et al.* (1988) found out that mangrove leaves exposed to direct sunlight had higher photosynthetic efficiencies when cultivated in 10 % seawater as compared to the leaves of plants cultivated in full-strength seawater. Reductions in F_v/F_m were also observed when the salinity effect was combined with a superimposed stress (Sharma and Hall 1989, Larcher *et al.* 1990, Jimenez *et al.* 1997). We found that at a high irradiance the HS-grown *K. candel* seedlings had reduced F_v/F_m possibly indicating photoinhibition. Additionally, LN-treatment exacerbated the reducing effect. The reduced N availability predisposes soybean leaves to photoinhibition (Kao and Forseth 1992).

The effects of N availability on P_N and Chl *a* fluorescence depended on salinity (Table 1, Figs. 1 and 2). With LS, rising N availability significantly increased P_N values. Hence, a higher F_v/F_m measured in HN than in LN leaves of LS-grown plants was probably due to the fact that the former had a higher P_N, thus managed to keep bigger number of oxidized Q_A molecules (a higher q_P value). Because the susceptibility of photosynthesis to photoinhibition increases with the level of steady-state reduction of Q_A (Osmond *et al.* 1993, Ottander *et al.* 1993), the HN-plants were less susceptible to photoinhibition than the LN-plants. As salinity went up, increasing N-availability had a limited effect on P_N and q_P. However, under HS, leaves of LN-plants had developed a higher capacity of thermal dissipation (a higher q_N) than the HN-plants. Thus, the higher reduction of F_v/F_m measured in LN- than in HN-leaves of HS-plants should be considered mainly due to the development of a higher photoprotection (a higher q_N) in the LN-leaves. Similar to this result, increases in q_N and decreases in F_v/F_m ratio were also found in nitrogen-deficient maize plants (Khamis *et al.* 1990).

The effects of increase in salinity on Chl *a* fluorescence were different between HN- and LN-plants. At HN, a higher P_N in LS-plants resulted in a higher q_P in these plants than in HS-plants (Table 1, Fig. 2). However, as the N availability declined, no significant effect of NaCl on effective quantum yield of PS2 and q_P was found, but the HS-plants had significantly higher q_N than the LS plants when their leaves were exposed to PFD>1100 μmol m^{-2} s^{-1}. Thus, an enhancement of thermal dissipative processes may contribute to a higher reduction of F_v/F_m in the leaves of HSLN plants than in those of LSLN plants.

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