Acta Physiol Plant (2014) 36:1903–1917
DOI 10.1007/s11738-014-1566-0

ORIGINAL PAPER

# Study on photosynthetic responses and chlorophyll fluorescence in *Rhizophora mucronata* seedlings under shade regimes

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Received: 8 October 2013 / Revised: 18 March 2014 / Accepted: 30 April 2014 / Published online: 24 May 2014


**Abstract** The photosynthetic performance of mangrove *Rhizophora mucronata* seedlings grown under seasonally full light (HL), 50 % shade (ML), and 80 % shade (LL) conditions was characterized by gas exchange, and chlorophyll fluorescence. The carboxylation efficiency significantly affected the seasonal change of the photosynthetic capacity. Temperature and light might have synergic effect on the carboxylation efficiency. The photosynthetic rate (PN) of *R. mucronata* seedlings under shade regimes, however, could not be attributed to variability in chlorophyll, $C_i$, ΦPSII, ETR or qP values but more to differences in carboxylation efficiency, $g_{max}$, and $E_{max}$. HL and ML plants had higher PN, $g_s$ and $E$ than the LL ones. Nevertheless, LL leaves exhibited low photoinhibition susceptibility. The high non-photochemical quenching in HL leaves may show that applied light intensity probably exceeded the photosynthetic capability. The findings indicate that ML treatments provided the best condition to obtain such carbon fixation capacity.

Communicated by Z. Gombos.

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**Keywords** Chlorophyll fluorescence · Mangrove · Photoinhibition · Photosynthesis · *Rhizophora mucronata* · Shade tolerance

**Abbreviations**

| | |
|---|---|
| $C_i$ | Intercellular $CO_2$ concentration |
| $E$ | Transpiration rate |
| $E_{max}$ | Maximum transpiration rate |
| ETR | Electron transport rate |
| $F_v/F_m$ | Ratio of variable to maximum chlorophyll fluorescence |
| $g_{max}$ | Maximum stomatal conductance |
| $g_s$ | Stomatal conductance |
| PAR | Photosynthetically active radiation |
| $P_{max}$ | Maximum photosynthetic rate |
| $P_N$ | Net photosynthetic rate |
| PSII | Photosystem II |
| qN | Non-photochemical quenching |
| qP | Photochemical quenching |
| SPAD | Soil plant analysis development |
| Vpdl | Vapor pressure deficit between the leaf and air |
| ΦPSII | Quantum yield of photosystem II |

## Introduction

Mangroves represent an important coastal ecosystem in the tropic area because of their high productivity and adaptation ability under various abiotic stresses. Subjects to daily, monthly, and annual variations in their physical environment, mangroves have a remarkable ability to survive with stress conditions (McLeod and Salm 2006). Especially light, salinity, and flooding are considered as the dynamic stressors in mangrove habitat.

Adaptation to shade is one of the causes of mangrove distribution patterns (Macnae 1969). Significant differences in the survival rates of the mangrove species were found depending on their intertidal positions and light exposition (Smith 1987). One hypothesis claimed that shade intolerance of mangrove seedlings was an additional stress on the ever-present stressor, salinity (Janzen 1985). Furthermore, the different light requirements among mangrove species indicated light-dependent responses of photosynthetic rate (Clough 1998) with different responses for each mangrove species (Kitao et al. 2003; Krauss and Allen 2003).

Mangroves belong to the $C_3$ plants that might show differences in photosynthetic capacity and sensitivity to environmental conditions for different species (Ball 1986). As regards light competition, gas exchange and chlorophyll fluorescence characteristics of mangrove *Avicennia marina* is typical of sun leaves (Ball and Critchley 1982). On the other hand, *Bruguiera sexangula* responded favorably to sunlight at low light level and is considered as relatively shade-tolerant species (Krauss and Allen 2003).

*Rhizophora mucronata* Lamk, "the intermediate gap-phase mangrove species", is found worldwide from East Africa and India through Asia as well as Indonesia to the Western Pacific, in wet tropical regions of Australia and in Mozambique and South Africa (Hoppe-Speera et al. 2011). In Indonesia, *R. mucronata* commonly found between zonation of *Avicennia* and *Bruguiera* (White et al. 1989; Whitten et al. 2000) occupies a gradient from low intertidal swamp margins with high insulation, to shaded sites at high water. *Rhizophora mucronata* had a role as main plant in the reforested thinned site in tropical coastal area (Srivastava et al. 1988) and produced more leaf litter than the reforested unthinned and natural sites (Wang'ondu and Virginia 2010). While thinning activity contributes on shading conditions, information of seedlings' adaptive capacity to shade regimes in relation to photosynthetic performances is essential to clarify both the mangrove zonation pattern and the growth model of *R. mucronata* in the restoration area.

Light or shade regimes were considered to affect not only photosynthetic rate, but also chlorophyll fluorescence. Exposure to excess irradiance can lead to photoinhibition, which is characterized by a light-dependent reduction in the fundamental quantum yield of photosynthesis and a loss of photosystem II (PSII) activity (Osmond 1994). So far, there is no specific information about chlorophyll fluorescence of *R. mucronata* seedlings under shade regimes.

The contrasting low- and high-shading areas will create varying combinations of light and temperature also. Temperature modification in gas exchange analysis could improve the accuracy of estimation of the net $CO_2$ fixation capacity (Okimoto et al. 2007). Ong et al. (1995) reported that the temperature on the top of the mangrove canopy was about 10 °C higher than at the ground surface. If a shaded leaf becomes exposed to full sunlight, does its temperature exceed the optimum for photosynthesis? Conversely, what happens with a leaf originally sunned, has the lowering temperature upon shading any advantage for its functioning? To answer such questions, we also investigated the photosynthetic responses of sunned and shaded leaves of *R. mucronata* seedling for 1 year, while the temperature was different at each month.

Seasonal information of photosynthetic rate and chlorophyll fluorescence in *R. mucronata* seedlings under shade regimes may contribute to a better understanding how environmental conditions govern photosynthetic capacity and thus, for better estimating mangrove productivity with photosynthetic growth model (Okimoto et al. 2008).

## Materials and methods

### Plant materials and growth conditions

Propagules of *R. mucronata* were collected from Galang Island (0°45′N, 104°15′E) in Batam District, Indonesia. Propagules were planted in the greenhouse with heating system at the Laboratory of Tropical Crop Improvement, Faculty of Agriculture, Saga University, Japan (33°14′N, 130°17′E) on June 2010. After 5 months, seedlings with 3–4 pairs of leaves were grown under full sunlight (HL), 50 % shading (ML) and 80 % shading (LL). Shade treatments were done by neutral density black nylon netting. During the experiment, seedlings were watered to ensure that drought did not confound experimental results.

Light intensities were measured on mid-day at July 20, 2012, a sunny cloudless day, and showed that the actual photosynthetically active radiation (PAR) was 1,728, 885, and 345 μmol photon $m^{-2}$ $s^{-1}$ for HL, ML and LL treatments, respectively. It showed that the shading level after 1-year treatment was still consistent at full sunlight, 50 and 80 % shading conditions. The monthly variation of air temperature in the greenhouse from August 2011 to July 2012, recorded hourly with a portable Thermo Recorder equipped with an external thermosensor (TR-50C, T and D co. Ltd., Nagano, Japan). The maximum, minimum and average temperature of each day were determined, and these daily values were averaged over a month to get the data points displayed in Fig. 1.

### Leaf gas exchange

The responses of mangrove seedling for leaf gas exchange to shade treatments were evaluated for 1 year from August 2011 to July 2012, beginning after seedlings had been

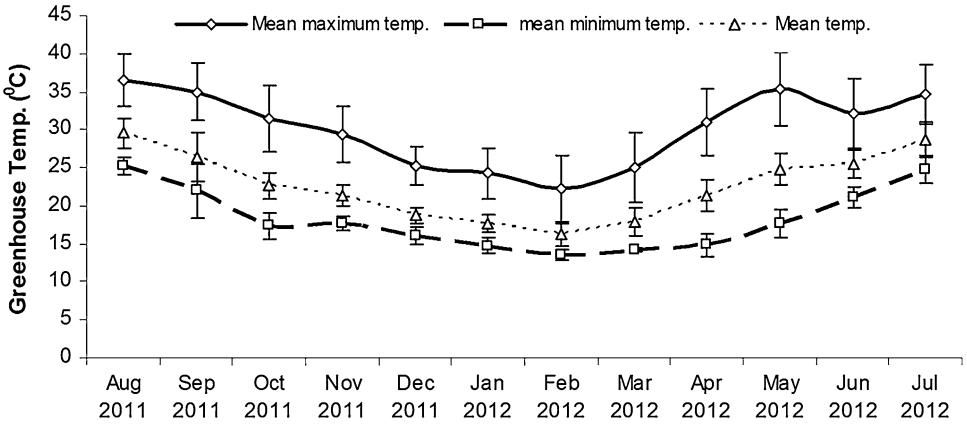



**Fig. 1** Mean monthly, mean monthly minimum, and mean monthly maximum of greenhouse air temperature during 1-year experiment. Values are means ± SD ($n$ = number of days in each months). Especially during cold months (December 2011–March 2012), the minimum greenhouse temperature was arranged more than 10 °C

exposed to their shading treatments for 8 months. Net photosynthetic rate ($P_N$), transpiration rate ($E$), stomatal conductance ($g_s$) and intercellular $CO_2$ concentration ($C_i$) were measured with a portable open-flow gas exchange system (LI-6400, Li-COR, Lincoln, NE, USA). Measurements were made at fully expanded leaves on sunny days from the morning (08:00 h, local time) until close to midday (11:00 h) only.

Photosynthetic rate under shade regimes was evaluated in relation to light intensity and temperature. In relation to light intensity, PAR value on leaf surfaces was automatically maintained in decreasing order from 1,000 to 0 μmol $m^{-2}$ $s^{-1}$ (1,000, 500, 250, 100, 50, 0 μmol $m^{-2}$ $s^{-1}$), and leaf temperature was controlled at 30 °C, vapor pressure deficit between the leaf and air (VpdL) was 1.7 $\pm$ 0.3 kPa, and $CO_2$ input was 370 μmol $mol^{-1}$. Furthermore, the effect of leaf temperature on photosynthetic rate was measured from 20 to 38 °C under PAR, VpdL and $CO_2$ input were 1,000 μmol $m^{-2}$ $s^{-1}$, 1.7 $\pm$ 0.3 kPa, and 370 μmol $mol^{-1}$, respectively. In order to minimize the temperature shock effect, the starting temperatures were different for each seasons, they were lower during cold months than hot months. Furthermore, the quantifying of photosynthetic rate as $C_i$ function was done by changing the $CO_2$ concentration at the leaf surface from 0 to 1,000 μmol $mol^{-1}$, under PAR 1,000 μmol $m^{-2}$ $s^{-1}$ and leaf temperature 30 °C.

## Chlorophyll fluorescence

Leaf chlorophyll fluorescence was measured with a modulated chlorophyll fluorometer (OS5-FL, OPTI-SCIENCES, USA) between 08:00 and 11:00 h, on the same leaves used for gas exchange analysis. The fluorescence parameters were obtained under both dark-adapted fluorescence and yield of energy conversion as described by Genty et al. (1989). In leaves submitted to darkness, readings were taken after 30-min dark adaptation using a leaf clip. Minimum fluorescence ($F_o$) was determined by a weak red light, and maximum fluorescence ($F_m$) was induced by a 0.8-s pulse of 2,000 μmol $m^{-2}$ $s^{-1}$ PAR. The steady-state fluorescence ($F_s$) was recorded, and a second saturating pulse was applied to determine the maximum light-adapted fluorescence ($F_{m'}$). A 685-nm light source equipped with OS5-FL was used for the illumination of leaf as actinic light. The actinic light was removed then the minimum fluorescence level in the light-adapted state ($F_{o'}$) was determined after 10 s of far red illumination. The following chlorophyll fluorescence parameters were calculated according to Genty et al. (1989) and Maxwell and Johnson (2000): quantum yield of photosystem II, $\Phi PSII = (F_{m'} - F_s)/F_{m'}$; maximum quantum efficiency of fluorescence PSII, $F_v/F_m = (F_m - F_o)/Fm$; photochemical quenching coefficient, $qP = (F_{m'} - F_s)/(F_{m'} - F_{o'})$; non-photochemical quenching, $qN = (F_m - F_{m'})/(F_m - F_{o'})$; and electron transport rate, $ETR = \Phi PSII \times PAR \times 0.5 \times 0.84$. PAR corresponds to the flux density of incident photosynthetically active radiation, 0.5 was a factor that accounts for the portioning of energy between PSII and PSI, and 0.84 was an average of the incident light absorbed by the leaf.

## Soil plant analysis development (SPAD) measurement

Soil plant analysis development value as representative of relative chlorophyll content was measured by using SPAD-Chlorophyll meter (SPAD 502, Minolta, Osaka, Japan). The utility of SPAD meter use is now widely accepted due to excellent correlation of SPAD 502 readings with chlorophyll content (Loh et al. 2002).

## Statistical analysis

All statistical tests were performed with Tukey HSD's test to detect the differences between means. Significant differences are reported as $P < 0.05$.

## Results

### Leaf morphology and SPAD value

Shade treatments affected *R. mucronata* leaf morphology. LL leaves were larger than HL and ML leaves. Leaf color of LL plants was dark green, while those of ML- and HL plants was green and light green, respectively (Fig. 2).

Soil plant analysis development readings being in tight correlation with chlorophyll content (Markwell et al. 1995) showed similar HL < ML < LL pattern for each month (Fig. 3). HL and ML leaves showed seasonal SPAD value variation and exhibited a slight minimum around February 2012. Furthermore, decreasing SPAD value of HL leaves also occurred in July 2012. The minimum SPAD value for LL leaves occurred in July 2012, but did not show significant seasonal variation.

### Effects of light intensity on $P_N$, $g_s$, $E$, and $C_i$

Variation of $P_N$ responses to light intensity at 30 °C of leaf temperature showed almost similar trends for all three treatments, increased simultaneously with PAR escalation until reaching their saturation point (Fig. 4).

The light responses of $P_N$, $g_s$ and $E$ were determined using the rectangular hyperbola model (Okimoto et al. 2008; Table 1):

$$P = \frac{I}{\alpha + \beta \cdot I} \quad (1)$$

where $P$ is $P_N$ of individual leaves at light intensity of $I$ (μmol photons $m^{-2}$ $s^{-1}$), then $\alpha$ and $\beta$ are coefficients to determine the convexity of the hyperbola. When used to model the conductance and transpiration responses, P was substituted to represent the $g_s$ and $E$ values in Eq. 1. Generally, the $P_N$, $g_s$ and $E$ of LL were lower than HL and ML leaves.

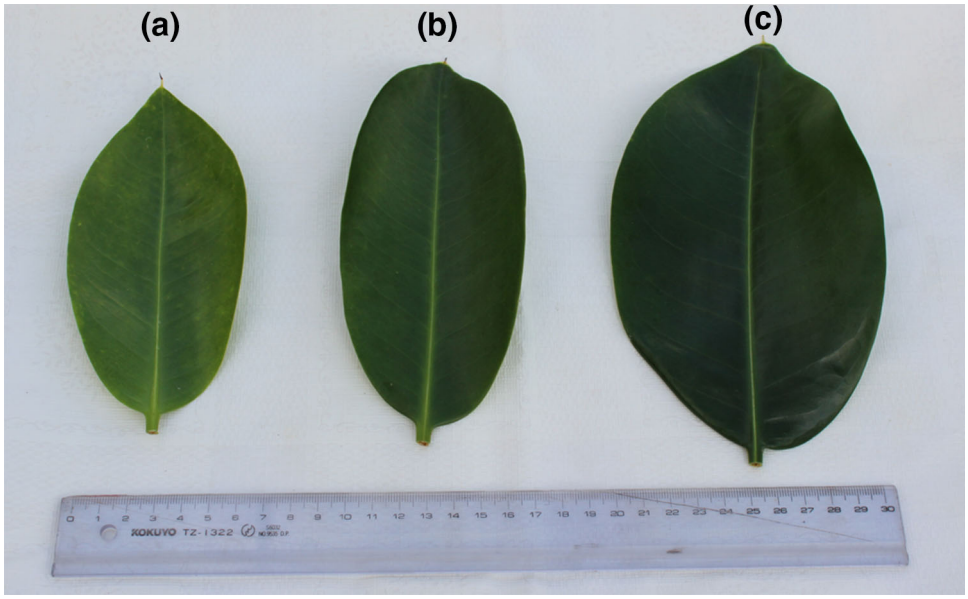



**Fig. 2** Leaves of *R. mucronata* from the various shade treatments: **a** full sunlight, **b** 50 % shade, **c** 80 % shade. They were collected on September 16, 2012

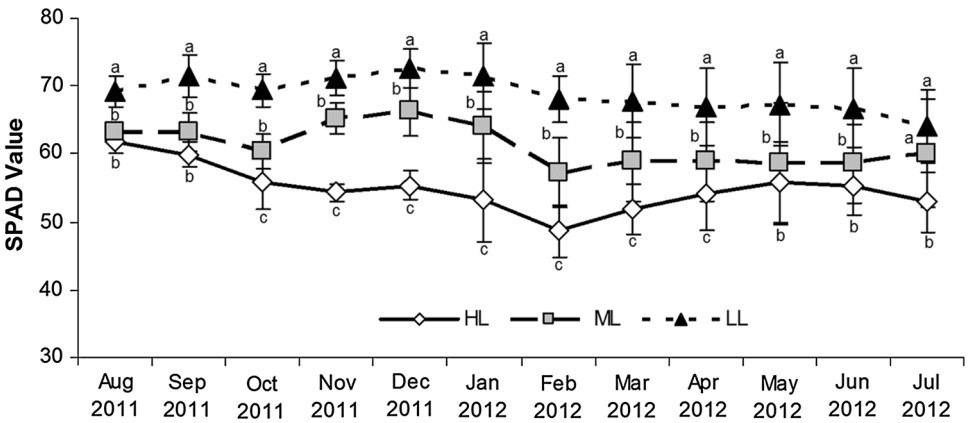



**Fig. 3** SPAD value in leaves of *R. mucronata* grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) conditions. Values are means ± SD ($n$ = 3–4 plants). Means in the same month, followed by *different letters* indicated significant differences between shade regimes ($P < 0.05$; Tukey HSD's test)

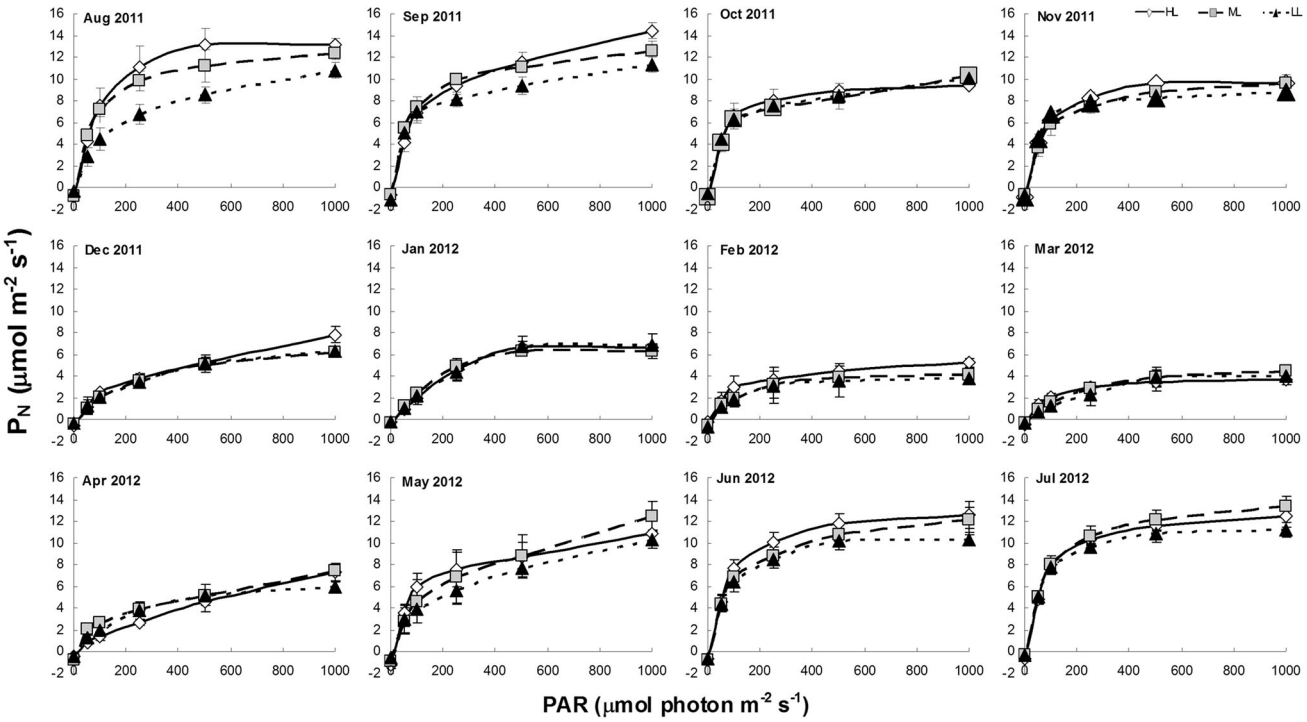



**Fig. 4** Response of net photosynthetic rate ($P_N$) to increasing photosynthetically active radiation (PAR) in the leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) conditions. They were measure at leaves temperature 30 °C. Values are mean ± SD ($n$ = 3–4 plants)

Equation 1 was used to determine maximum photosynthetic rate ($P_{max}$), maximum stomatal conductance ($g_{max}$), and maximum transpiration rate ($E_{max}$) at light saturation conditional (Table 1). The light saturation points of all treatments were commonly at PAR level around 1,000 μmol photons $m^{-2}$ $s^{-1}$. $P_N$, $g_s$ and $E$ responses to light during hot and sunny months (June–September) tended to increase rapidly up to PAR 100 μmol $m^{-2}$ $s^{-1}$, had high values and wide gap value between shading treatments at saturation point. On the other side, during cold months (December–March) they were characterized with rapid increasing up to PAR about 250 μmol $m^{-2}$ $s^{-1}$, low values and no significance difference at saturation point (Fig. 4).

Under light saturation, $P_{max}$ showed a positive correlation with $g_{max}$ and $E_{max}$ (Fig. 5). The highest values of $g_{max}$ and $E_{max}$ showed similar trends, there were LL < ML < HL, respectively. Lower rates of $g_{max}$ and $E_{max}$ for LL leaves probably restricted $P_{max}$. We found that although the highest value of $g_{max}$ and $E_{max}$ of ML was lower than HL, but their highest $P_{max}$ value had similar tendency.

## Effect of temperature on photosynthesis

The quadratic curves were fitted to describe the temperature responses of $P_N$ (Fig. 6). The results showed that relationship between $P_{max}$ and leaf temperature indicated a broad peak for different seasons. During mid-high temperature months between August–November 2011 and May–July 2012, $P_{max}$ was obtained at leaf temperature 29–34 °C, and between 23 and 29 °C during cold months (December 2011–April 2012). $P_{max}$ for the temperature responses of HL (14.9 μmol $m^{-2}$ $s^{-1}$) and LL (12.0 μmol $m^{-2}$ $s^{-1}$) occurred on September 2011 at leaf temperature 32 °C, while ML (13.8 μmol $m^{-2}$ $s^{-1}$) ensued on July 2012 at 33 °C.

$P_N$ shows seasonal variation while leaf temperature was set at 30 °C correlating with the pre-condition temperature (high $P_N$ in the hot months, and lower in the colder ones). During the hot months, LL leaves sustained a better photosynthetic performance at leaf temperature 25 °C than HL and ML leaves (Fig. 7).

## Effect of $C_i$ on photosynthesis

The carboxylation efficiency relating with Rubisco activity can be estimated as the initial slope of the response $P_N$ to $C_i$ (Ku and Edwards 1977; Sage and Reid 1994). The initial slope of $P_N$ ($C_i$) curve is calculated and derived from Eq. 1, while $C_i$ tends to zero, i.e.,

**Table 1** The values of $P_{max}$, $g_{s-max}$, and $E_{max}$ at saturating level of PAR 1,000 μmol photons $m^{-2}$ $s^{-1}$ and leaf temperature 30 °C in leaves of *R. mucronata* grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) conditions

| Code | Month | Equation | | | $P_{max}$ | $g_{s-max}$ | $E_{max}$ |
|---|---|---|---|---|---|---|---|
| | | $P_N$ | $g_s$ | $E$ | | | |
| HL | Aug 2011 | $P_N = I/(4.85 + 0.07I)$ | $g_s = I/(82.08 + 2.44I)$ | $E = I/(2.74 + 0.20I)$ | 13.18 | **0.40** | **4.93** |
| | Sep 2011 | $P_N = I/(12.23 + 0.06I)$ | $g_s = I/(325.56 + 2.81I)$ | $E = I/(15.95 + 0.21I)$ | **14.42** | 0.32 | 4.43 |
| | Oct 2011 | $P_N = I/(6.45 + 0.10I)$ | $g_s = I/(126.62 + 5.89I)$ | $E = I/(8.31 + 0.38I)$ | 9.44 | 0.17 | 2.58 |
| | Nov 2011 | $P_N = I/(7.45 + 0.09I)$ | $g_s = I/(152.773 + 4.76I)$ | $E = I/(7.89 + 0.34I)$ | 10.16 | 0.20 | 2.87 |
| | Dec 2011 | $P_N = I/(43.95 + 0.08I)$ | $g_s = I/(6213.07 + 3.88I)$ | $E = I/(204.92 + 0.51I)$ | 7.82 | 0.10 | 1.40 |
| | Jan 2012 | $P_N = I/(20.50 + 0.13I)$ | $g_s = I/(4123.21 + 8.36I)$ | $E = I/(118.21 + 0.73I)$ | 6.87 | 0.08 | 1.18 |
| | Feb 2012 | $P_N = I/(26.30 + 0.16I)$ | $g_s = I/(1764.07 + 9.04I)$ | $E = I/(45.07 + 0.81I)$ | 5.25 | 0.09 | 1.17 |
| | Mar 2012 | $P_N = I/(23.51 + 0.24I)$ | $g_s = I/(1742.51 + 9.56I)$ | $E = I/(86.8 + 0.81I)$ | 3.74 | 0.09 | 1.12 |
| | Apr 2012 | $P_N = I/(81.19 + 0.06I)$ | $g_s = I/(3260.60 + 11.34I)$ | $E = I/(615.12 + 0.38I)$ | 7.34 | 0.07 | 1.00 |
| | May 2012 | $P_N = I/(9.72 + 0.083I)$ | $g_s = I/(112.97 + 3.67I)$ | $E = I/(12.49 + 0.28I)$ | 10.83 | 0.26 | 3.42 |
| | Jun 2012 | $P_N = I/(5.66 + 0.07I)$ | $g_s = I/(11.00 + 6.05I)$ | $E = I/(27.00 + 0.46I)$ | 12.54 | 0.16 | 2.05 |
| | Jul 2012 | $P_N = I/(5.85 + 0.07I)$ | $g_s = I/(92.61 + 3.93I)$ | $E = I/(16.43 + 0.0.25I)$ | 12.49 | 0.25 | 3.75 |
| ML | Aug 2011 | $P_N = I/(6.73 + 0.07I)$ | $g_s = I/(129.04 + 3.28I)$ | $E = I/(3.10 + 0.25I)$ | 12.33 | 0.29 | 3.95 |
| | Sep 2011 | $P_N = I/(6.73 + 0.07I)$ | $g_s = I/(82.40 + 3.24I)$ | $E = I/(4.13 + 0.24I)$ | 12.33 | **0.30** | **4.10** |
| | Oct 2011 | $P_N = I/(10.23 + 0.09I)$ | $g_s = I/(55.86 + 5.78I)$ | $E = I/(2.22 + 0.38I)$ | 10.28 | 0.17 | 2.62 |
| | Nov 2011 | $P_N = I/(9.78 + 0.09I)$ | $g_s = I/(293.92 + 4.26I)$ | $E = I/(16.41 + 0.28I)$ | 9.64 | 0.22 | 3.37 |
| | Dec 2011 | $P_N = I/(41.28 + 0.12I)$ | $g_s = I/(819.29 + 8.41I)$ | $E = I/(111.38 + 0.60I)$ | 6.20 | 0.11 | 1.41 |
| | Jan 2012 | $P_N = I/(14.93 + 0.13I)$ | $g_s = I/(1934.98 + 11.57I)$ | $E = I/(57 + 0.58I)$ | 6.87 | 0.07 | 1.57 |
| | Feb 2012 | $P_N = I/(22.82 + 0.22I)$ | $g_s = I/(359.04 + 12.69I)$ | $E = I/(81.37 + 1.37I)$ | 4.13 | 0.08 | 0.69 |
| | Mar 2012 | $P_N = I/(39.52 + 0.19I)$ | $g_s = I/(3290.72 + 23.11I)$ | $E = I/(55.79 + 1.01I)$ | 4.45 | 0.04 | 0.94 |
| | Apr 2012 | $P_N = I/(41.32 + 0.09I)$ | $g_s = I/(1194.92 + 11.34I)$ | $E = I/(78.20 + 0.67I)$ | 7.48 | 0.08 | 1.34 |
| | May 2012 | $P_N = I/(21.70 + 0.06I)$ | $g_s = I/(287.65 + 6.72I)$ | $E = I/(56.29 + 0.51I)$ | 12.48 | 0.14 | 1.77 |
| | Jun 2012 | $P_N = I/(10.18 + 0.07I)$ | $g_s = I/(20.00 + 6.50I)$ | $E = I/(40.54 + 0.33I)$ | 12.10 | 0.15 | 2.70 |
| | Jul 2012 | $P_N = I/(6.382 + 0.07I)$ | $g_s = I/(114.04 + 3.69I)$ | $E = I/(10.68 + 0.25I)$ | **13.37** | 0.26 | 3.84 |
| LL | Aug 2011 | $P_N = I/(18.45 + 0.07I)$ | $g_s = I/(870.52 + 6.26I)$ | $E = I/(59.80 + 0.341I)$ | 10.82 | 0.14 | 2.50 |
| | Sep 2011 | $P_N = I/(11.54 + 0.08I)$ | $g_s = I/(13.00 + 4.60I)$ | $E = I/(0.75 + 0.29I)$ | **11.35** | **0.22** | **3.44** |
| | Oct 2011 | $P_N = I/(5.19 + 0.10I)$ | $g_s = I/(107.65 + 6.28I)$ | $E = I/(0.6 + 0.43I)$ | 9.88 | 0.16 | 2.32 |
| | Nov 2011 | $P_N = I/(5.32 + 0.11I)$ | $g_s = I82.27 + 6.34I)$ | $E = I/(9.55 + 0.37I)$ | 8.82 | 0.16 | 2.63 |
| | Dec 2011 | $P_N = I/(36.61 + 0.12I)$ | $g_s = I/(1748.05 + 9.16I)$ | $E = I/(175.2 + 0.61I)$ | 6.34 | 0.09 | 1.27 |
| | Jan 2012 | $P_N = I/(14.93 + 0.13I)$ | $g_s = I/(1175.72 + 13.23I)$ | $E = I/(140.17 + 0.60I)$ | 6.87 | 0.07 | 1.35 |
| | Feb 2012 | $P_N = I/(17.51 + 0.25I)$ | $g_s = I/(1284.39 + 10.33I)$ | $E = I/(157.69 + 1.08I)$ | 3.80 | 0.09 | 0.81 |
| | Mar 2012 | $P_N = I/(50.41 + 0.20I)$ | $g_s = I/(728.15 + 9.52I)$ | $E = I/(711.87 + 0.85I)$ | 4.07 | 0.10 | 0.64 |
| | Apr 2012 | $P_N = I/(32.26 + 0.13I)$ | $g_s = I/(887.56 + 11.37I)$ | $E = I/(111.15 + 0.70I)$ | 6.01 | 0.08 | 1.23 |
| | May 2012 | $P_N = I/(26.88 + 0.07I)$ | $g_s = I/(395.25 + 8.37I)$ | $E = I/(37.76 + 0.61I)$ | 10.35 | 0.11 | 1.54 |
| | Jun 2012 | $P_N = I/(6.78 + 0.09I)$ | $g_s = I/(173.69 + 10.76I)$ | $E = I/(245.45 + 0.51I)$ | 10.33 | 0.09 | 1.32 |
| | Jul 2012 | $P_N = I/(4.41 + 0.09I)$ | $g_s = I/(192.88 + 4.98I)$ | $E = I/(14.68 + 0.33I)$ | 11.22 | 0.19 | 2.90 |

The highest values of $P_{max}$, $g_{s-max}$ and $E_{max}$ for each treatments are indicated in bold numbers. The functions were fitted to the points up to the maximum value for $P_N$, $g_s$ and $E$ at the saturation value based on Eq. 1

$$P = \frac{I}{\alpha + \beta \cdot I}$$
$$P' = \frac{\alpha + \beta \cdot I - \beta \cdot I}{(\alpha + \beta \cdot I)^2}, \text{ and while } I \text{ toward zero} \quad (2)$$
$$P' = \frac{\alpha}{\alpha^2}$$
$$P' = \frac{1}{\alpha}$$

where $P'$, $I$ and α are initial slopes of $P_N$ ($C_i$) curve, intercellular $CO_2$ concentration and first coefficients to determine the convexity of the hyperbola, respectively. The carboxylation efficiency implied increase in photosynthetic rate achieved per unit increase in $CO_2$ at the site of $CO_2$ fixation. Furthermore, maximum photosynthetic rate responses to $C_i$ ($P_{max} - C_i$) that represent the capacity of leaf photosynthesis can be also determined from Eq. 1, while $C_i$ becomes infinity, i.e.,

$$P = \frac{I}{\alpha + \beta \cdot I}$$
$$\frac{1}{P} = \frac{\alpha}{I} + \beta, \text{ and while } I \text{ becomes } \infty \quad (3)$$
$$P_{max-c_i} = \frac{1}{\beta}$$

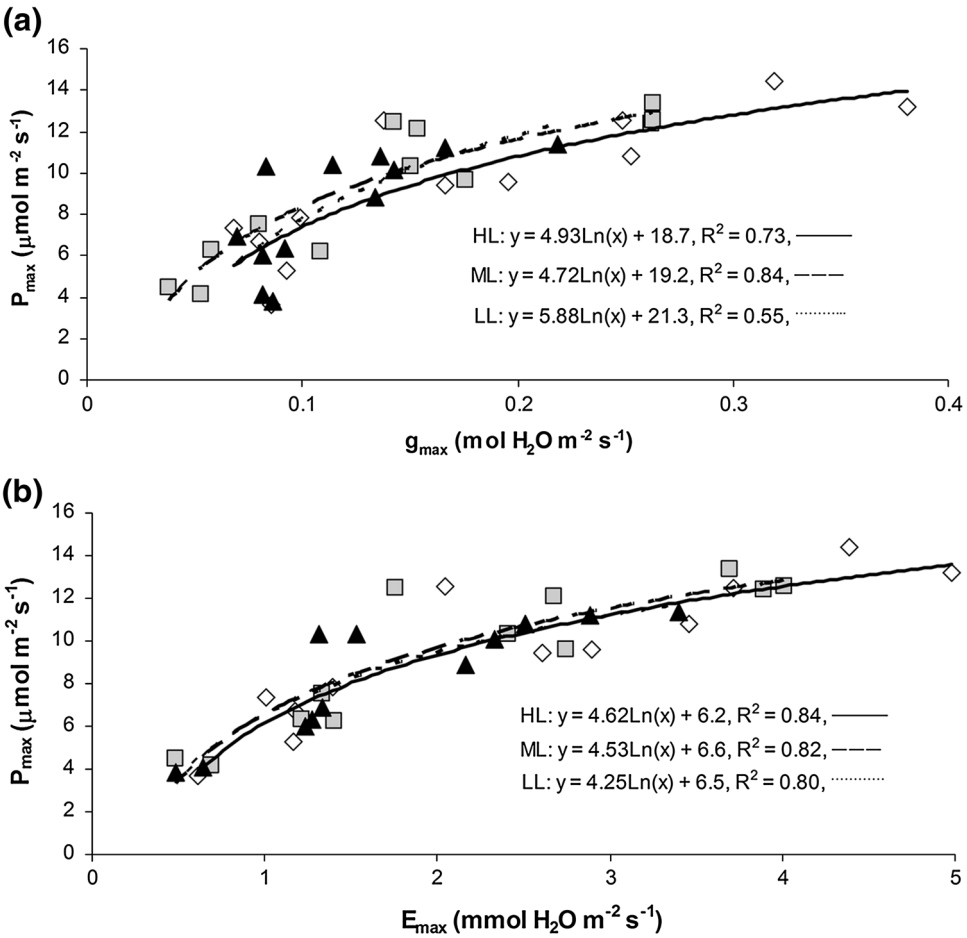



**Fig. 5** Maximum photosynthetic rate ($P_{max}$) as a function of **a** maximum stomatal conductance ($g_{max}$) and **b** maximum transpiration rate ($E_{max}$) for *R. mucronata* seedlings grown under full sunlight (*diamonds* and *solid lines*), 50 % shade (*squares* and *dash lines*), and 80 % shade (*triangles* and *dotted lines*). Data plotted from monthly value of $P_{max}$, $g_{max}$ and $E_{max}$ at PAR 1,000 μmol photon m$^{-2}$ s$^{-1}$ and leaf temperature 30 °C

where $P_{max}-C_i$ is the maximum photosynthetic rate response to $C_i$, and $\beta$ is second coefficient determining the convexity of the hyperbola. Figure 8 shows that initial slope of $P_N$ ($C_i$) had similar seasonal variation as $P_{max}-C_i$. Both $P'$ and $P_{max}-C_i$ during hot months were higher than in the cold months. This tendency may mean that seasonal change of leaf photosynthetic capacity is controlled by carboxylation efficiency. Since the initial slopes in LL leaves were somewhat lower in the higher temperature part of the year (from April to August) than in the HL and ML leaves, which may indicate that temperature and light have synergic effect on the initial slopes.

### Chlorophyll fluorescence

The seasonal variation of quantum yield of PSII (ΦPSII) and electron transport rate (ETR) measured after 30 min exhibited the same seasonal variations as the other photosynthetic parameters. The ΦPSII and ETR decreased from August 2011 to February 2012, then increased from March until July 2012. Their lowest values occurred on February 2012. Generally, light intensity had no significant effect either on the ΦPSII or ETR (Fig. 9).

Photochemical quenching (qP) is a ratio of light energy used in the transfer of photochemical electrons to total light energy captured by antenna pigment and non-photochemical quenching (qN) reflects a ratio of light energy consumed by heat to the total light energy (Zhou et al. 2010). The qP values showed a slight seasonal variation being higher between April and November than in the cold months (December–March) (Fig. 10a). Unexpectedly, the qP value for HL was high in February 2012, whereas the photosynthetic rate was low (Table 1). Furthermore, qN values of HL leaves exhibited slightly higher in February 2012 as compared with other months (Fig. 10b).

A reduction in the ratio of variable to maximum chlorophyll fluorescence ($F_v/F_m$) can be used as an indication of photoinhibition (Björkman and Demmig 1987; Robakowski 2005). HL and ML leaves showed seasonal $F_v/F_m$ ratio variation and exhibited a significant decreasing in February and March 2012 (Fig. 11).

## Discussion

The results showed significantly increased SPAD values ($P < 0.05$) and leaf sizes while in plants exposed to 50 and

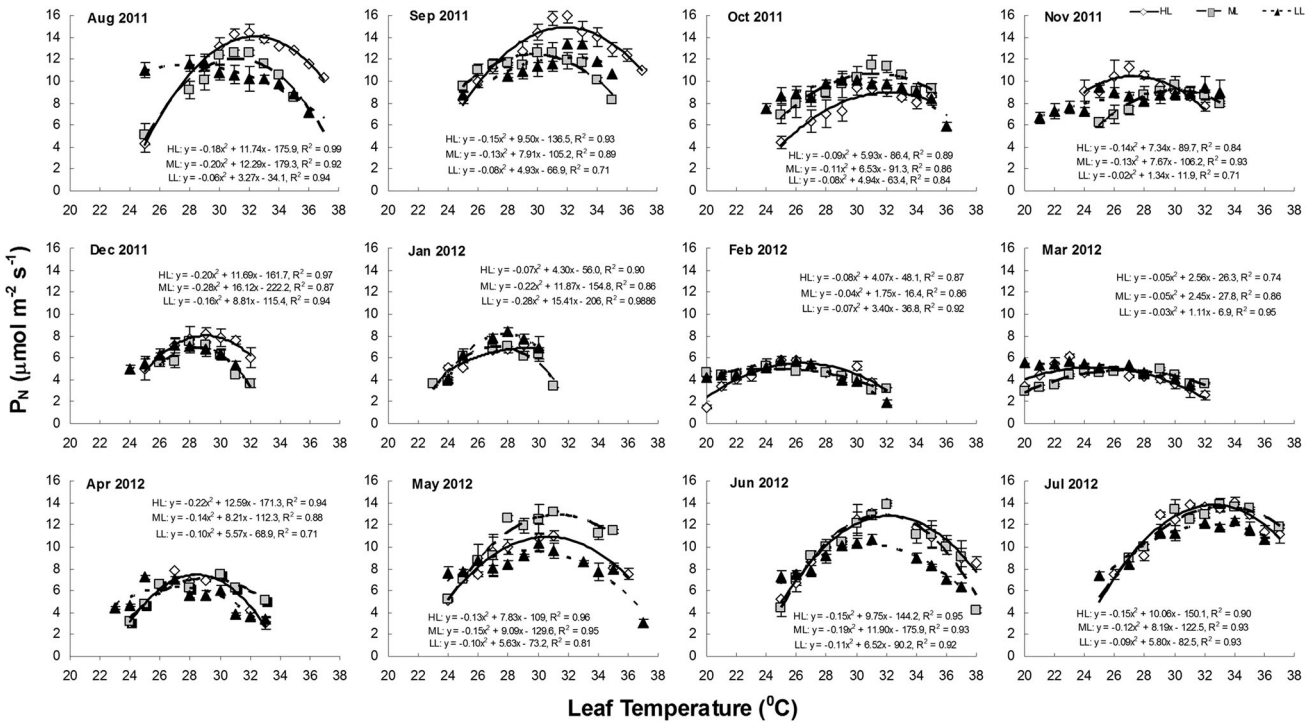



**Fig. 6** Response of net photosynthetic rate ($P_N$) to increasing leaf temperature *R. mucronata* seedlings grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) conditions. They were measured at PAR 1,000 μmol photon $m^{-2}$ $s^{-1}$. Values are mean ± SD ($n$ = 3–4 plants)

80 % shading (Figs. 2, 3). These results indicate the strategy of *R. mucronata* seedlings to adapt high light intensities: HL seedlings decreased their light absorption by reducing chlorophyll content and leaf area; in contrast, LL seedlings increased their light absorption by rising their leaf area and chlorophyll content. Previous studies have shown that plants grown under shaded conditions were noted to increase their pigment density per unit leaf area (Wittman et al. 2001; Xu et al. 2009), to optimize their height, leaf area, crown extension and leaf arrangement to get the best use of light (Paquette et al. 2007; Huang et al. 2011). When growing in a high-light environment, avoidance of light absorption, e.g., through low chlorophyll contents, played a crucial role in protecting the photosynthetic apparatus of leaves (Adams et al. 2004). We have also found a decoloring symptom with lower SPAD value of HL and ML leaves that must have been caused mainly by low temperature in February 2012. Decoloring may occur as a consequence of the combined effects of high-incident PAR and low temperature (Close et al. 1999). Especially for HL and ML leaves of *R. mucronata*, these results were in agreement with Kao et al. (2004) findings, which showed that leaves of mangrove *Avicennia marina* during low temperature at 15 °C had a greater reduction in chlorophyll content rather than 30 °C. On the other side, LL leaves had no decoloring symptom during low temperature, it was almost similar with no significance decreasing the chlorophyll content of mangrove *Kandelia candel* grown either at 30 or 15 °C (Kao et al. 2004). Although LL exhibited a significantly reduced SPAD value in July, this value was still higher than those of the HL and LL leaves in the same period (Fig. 3). We suggest the slight minimum SPAD value of LL leaves in July 2012 to regard as a LL-protection mechanism. The reduction of photosynthetic pigments could be seen as a protection mechanism as it would mitigate the capacity of the leaf to absorb incident radiation and therefore decreases the amount of excess excitation energy that has to be dissipated (Burritt and Mackenzie 2003).

A raise in total chlorophyll increased the gas exchange (Evans 1989), as shown in mangrove *A. marina* and *Hibiscus tiliaceus* (Naidoo et al. 2002). However, this study has been unable to demonstrate that higher total chlorophyll had high $P_N$ in *R. mucronata* seedlings under shade regimes. The result showed that the $P_N$ of LL was lower than HL and ML leaves (Fig. 4). We found that under light saturating conditions, $g_{max}$ and $E_{max}$ showed similar trends, they are LL < ML < HL, respectively (Fig. 5; Table 1). It described that the $P_{max}$ of *R. mucronata* seedlings was more influenced by $g_{max}$ and $E_{max}$ rather than chlorophyll content. The circulation of $CO_2$ is determined by stomatal density, size, and conductance

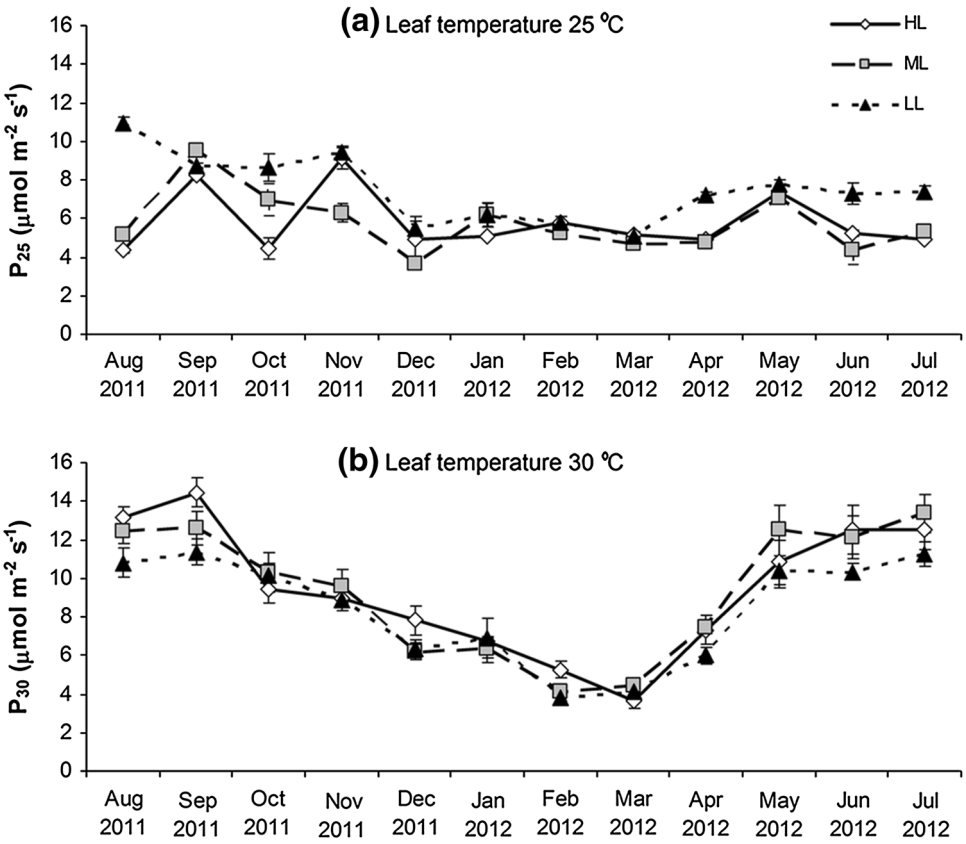



**Fig. 7** Net photosynthetic rate ($P_N$) of *R. mucronata* seedlings grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) at **a** leaf temperature 25 °C and **b** 30 °C. They were measured at PAR 1,000 µmol photon $m^{-2}$ $s^{-1}$. Values are mean ± SD ($n$ = 3–4 plants)

(Xuan et al. 2011), and among of those factors, stomatal conductance is the most prominent (Putra et al. 2012). Cheeseman et al. (1997) found that the relationship between net $CO_2$ assimilation and $g_s$ in mangrove *Rhizophora stylosa* was significant and positive while measured under intermediate temperature and high light. Lower rates of $g_{max}$ for LL leaves probably restricted the maximum photosynthetic rate, similarly as shown at "the shade tolerant mangrove species", *Bruguiera sexangula* (Krauss and Allen 2003). High stomatal conductance was followed by increased transpiration rate. The positive relationships between $P_N$, $g_s$ and $E$ were also found at mangroves seedlings of *R. stylosa* grown under light levels (Kitaya et al. 2002). Moreover, ability of ML leaves to achieve high $P_{max}$ in lower $g_{max}$ and $E_{max}$ compared with HL leaves indicates ML effectiveness and also chance to conserve water in better level. It will be useful while ML seedlings adapt with saline condition.

We found that the light saturation point of all treatments were commonly at PAR level around 1,000 µmol photons $m^{-2}$ $s^{-1}$. These results were higher than mangrove *B. sexangula* and similar with *A. marina.* The finding of Krauss and Allen (2003) estimated that light saturation point of *B. sexangula* seedlings is usually below 500 µmol photons $m^{-2}$ $s^{-1}$ under both LL and HL conditions. The assimilation rates of *A. marina*, "the sunlit mangrove species" became light saturated at approximately 1,000 µmol photons $m^{-2}$ $s^{-1}$ in leaves from shade condition and high-light regime (Ball and Critchley 1982). It can therefore be assumed that *R. mucronata* leaves are more a sunny leaf type while compared with those of *B. sexangula.* This finding corroborates the idea of Kitao et al. (2003) who suggested that within intermediate gap-phase species, *Rhizophora* prefers more sun-lit sites than *Bruguiera*.

Our finding showing different characteristics of $P_N$ responses of *R. mucronata* leaves to light intensity (Fig. 4) in the hot (June–September) and in the cold (December–March) months emphasized the role of temperature for mangrove seedling growth and photosynthetic performances. Low temperature clearly modified the passage of light response curves during cold months compared with hot months.

Photosynthesis of mangroves has been indicated to be highly sensitive to leaf temperature (Andrews et al. 1984; Ball et al. 1988). In view of the ecological distribution of plants, it was necessary to explain the temperature response curve of photosynthesis (Agata et al. 1985), and also could improve the accuracy of estimation of $CO_2$ fixation capacity by mangrove (Okimoto et al. 2007). Moore et al. (1973) reported that $P_{max}$ of mangrove *Rhizophora* and *Laguncularia* was obtained at leaf temperature near or below 25 °C. In contrast, some latter reports indicate that

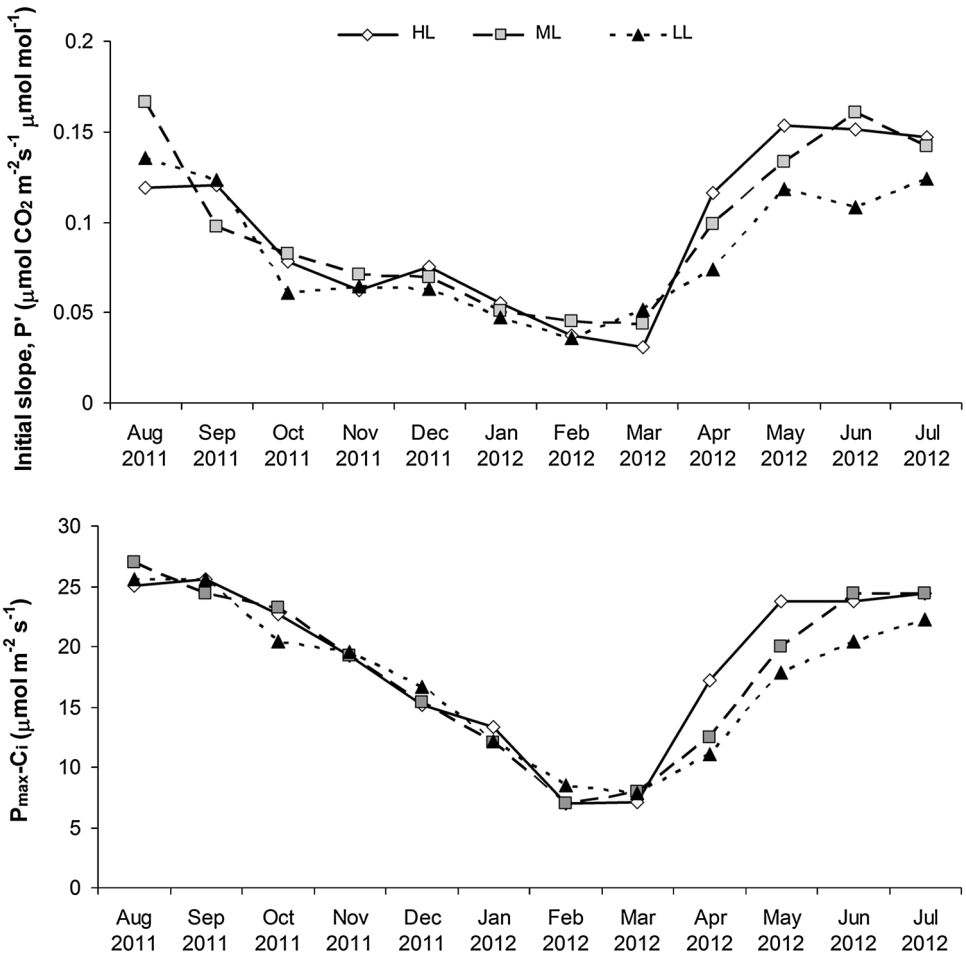



**Fig. 8** Monthly pattern of initial slope ($P'$) and maximum photosynthetic rate responses to $C_i$ ($P_{max}$ $-C_i$) of *R. mucronata* seedlings grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL). They were measured at leaves temperature 30 °C PAR 1,000 µmol photon $m^{-2}$ $s^{-1}$. The values of $P'$ and $P_{max}$ $-C_i$ were calculated with Eqs. 2 and 3, respectively

the relationship between the net photosynthetic rate and leaf temperature indicated a wide peak between 29 and 34 °C (Okimoto et al. 2007). Our finding showed that relationship between $P_{max}$ and leaf temperature indicated a broad peak, which was dependent on the pre-condition temperature. At high pre-condition temperatures between August–November 2011 and May–July 2012, $P_{max}$ was obtained between 29 and 34 °C leaf temperatures, but at lower (23–29 °C) leaf temperatures in the other months (Fig. 6). Furthermore, the effect of leaf temperature on $P_N$ shows seasonal variation only in those letters which were set at 30 °C correlating with the pre-condition temperature. During the hot months, we found that LL leaves sustained a better photosynthetic performance while leaf temperature was set at low temperature, 25 °C, as compared to HL and ML leaves (Fig. 7). Some studies have found that the optimum temperature for plant photosynthesis depended strongly on their growth temperature (Sawada and Miyachi 1974; Kao et al. 2004). The temperature is lower in deep-shade areas than in the sun-exposed ones; thus, LL seedlings exhibited better photosynthetic performance at lower temperatures.

Sharkey (1985) pointed out that the rates of photosynthesis were a function of both the stomata responses to allow carbon dioxide to penetrate the leaf and the biochemical capacity to fix $CO_2$. Change in the shape of the $P_N$ ($C_i$) curve was not only beneficial to indicate variability in the capacity for photosynthesis, but also elucidate which regions of photosynthetic biochemistry are sensitive to environment (Ball 1986). Initial slope of the response of $P_N$ to $C_i$ could be correlated to the in vivo assessment of biochemical components of leaf photosynthesis such as ribulose–bisphosphate carboxylase (rubisco) activity (Caemmerer and Farquhar 1981). Furthermore, maximum photosynthetic rate responses to $C_i$ are beneficial to indicate the capacity or potential of leaf photosynthesis. As shown in Fig. 8, the similar seasonal pattern of $P'$ and $P_{max}$ $-C_i$ suggested that the potential photosynthesis of *R. mucronata* leaves was strongly affected by carboxylation efficiency. Both of them were higher over the hot months as compared with the cold ones. In contrast to Sage and Reid (1994) who reported that the initial slope $P_N$ ($C_i$) was only slightly affected by temperature, we found that seasonal variation of temperature significantly affected $P'$ and

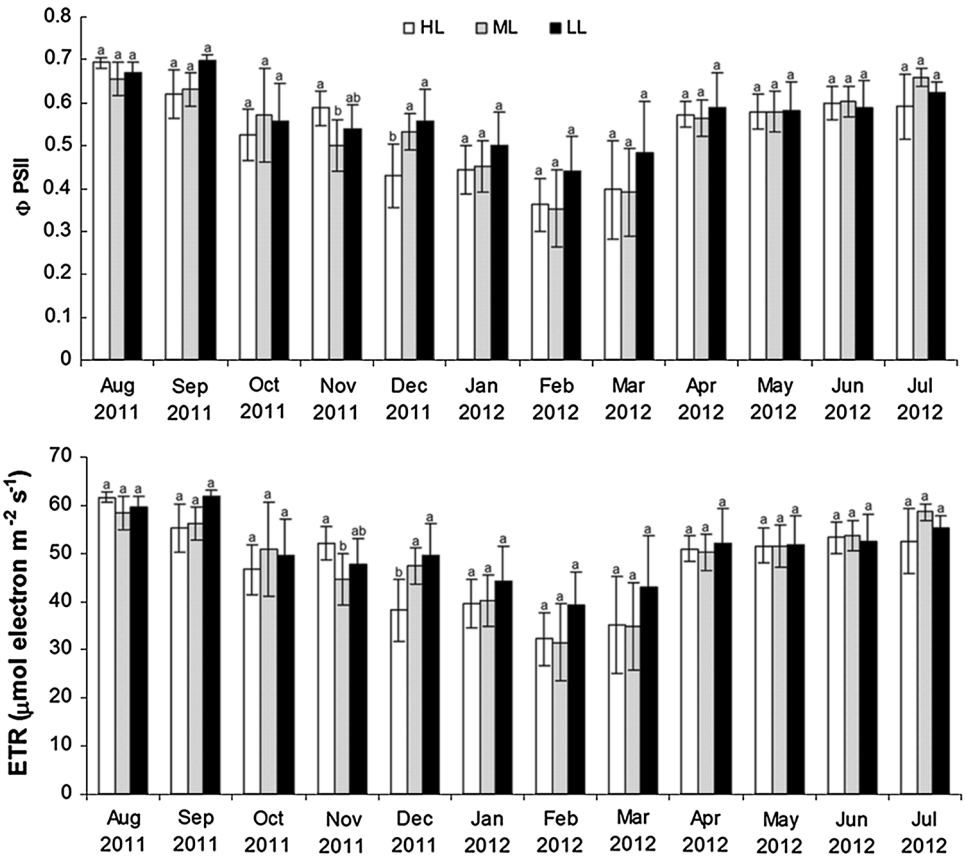


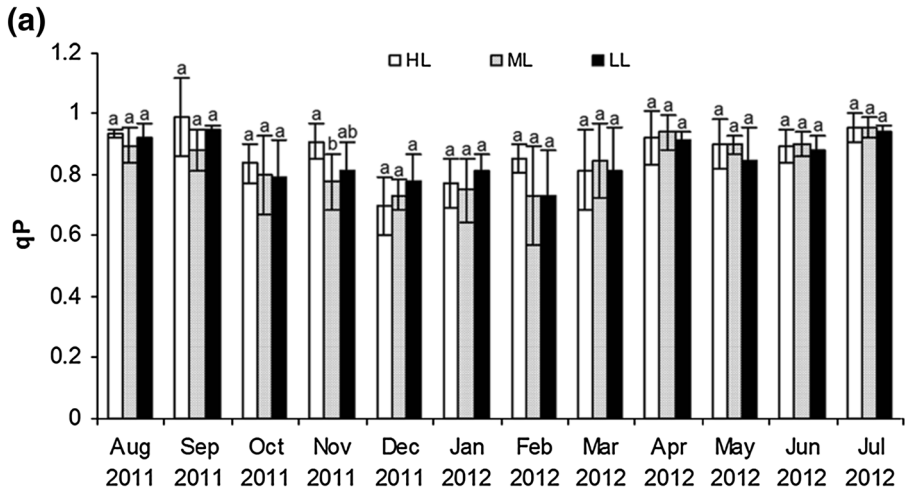

**Fig. 9** The quantum yield of PS II (ΦPSII) and electron transport rate (ETR) after 30-min dark adaptation at leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) conditions. Values are mean + SD ($n = 3$–4 plants). Means in the same month, followed by *different letters* indicated significant differences between shade regimes ($P < 0.05$; Tukey HSD's test)

$P_{max}-C_i$. This result is in agreement with that of Campbell et al. (2005) whose findings showed that increasing temperature increased the initial slope and the maximum rate of assimilation. During hot months, the low initial slope of LL leaves also indicated lower $P_N$ and $P_{max}-C_i$ in LL leaves as compared with HL and ML leaves. This result suggests that the carboxylation efficiency of *R. mucronata* leaves is also influenced by shade regimes. Sage and Reid (1994) reported that the changes in the content of the major photosynthetic constituent (PSII, ATP synthase, rubisco) occur with the greatest rate of adjustment after long-term acclimation to light regimes.

ΦPSII is the proportion of absorbed energy being used in photochemistry (Maxwell and Johnson 2000) that represents the efficiency of energy conversion of open PSII (Schreiber et al. 1994), and ETR represents the relative quantity of electron passing through PSII during steady-state photosynthesis (Tezara et al. 2003). Light intensity had no significance effect either on the ΦPSII or ETR. However, the reduction of ΦPSII and ETR for all treatments was found mainly during the cold months (Fig. 9). Lowering the temperature generally reduces the metabolic rates and can, therefore, limit the sinks for the absorbed excitation energy, particularly $CO_2$ fixation (Alam et al. 2005). A reduction in chlorophyll fluorescence in response to low temperature has also been observed in mangrove *K. candel* and *A. marina* (Kao et al. 2004). Furthermore, the combination of low temperature-high light intensity conditional during cold months might accelerate the damage to the photosynthetic apparatus (Alves et al. 2002).

The high qP values for all treatments during hot months are useful to sustain the high photochemical capacity. The similar patterns of the highest qP and $P_{max}$ value for each treatments that occurred on same months (Fig. 10a; Table 1) demonstrate the contribution of qP to $P_{max}$ achievement level. The response of qP represented the openness of PSII centers (Kitao et al. 2003), and high qP was beneficial for the separation of electric charge in reaction center (Dai et al. 2009). Furthermore, the high qP value of HL leaves on February 2012, whereas the low $P_N$ might indicate abnormal conditional because of photodamage. Although the mechanism is not clear, during low temperature on cold months, it was possible that photochemical quenching was not affected by temperature. Normally, a higher $P_N$ resulted a higher qP in plants (Kao and Tsai 1999).

Moreover, the high qN value of HL leaves on February 2012 (Fig. 10b) represented that the using of light energy probably already exceed photosynthetic capability and also level of heat dissipation. qN reflects the amount of energy dissipated by non-photochemical quenching by plants (Liu et al. 2007). While photosynthesis is incapable of using all



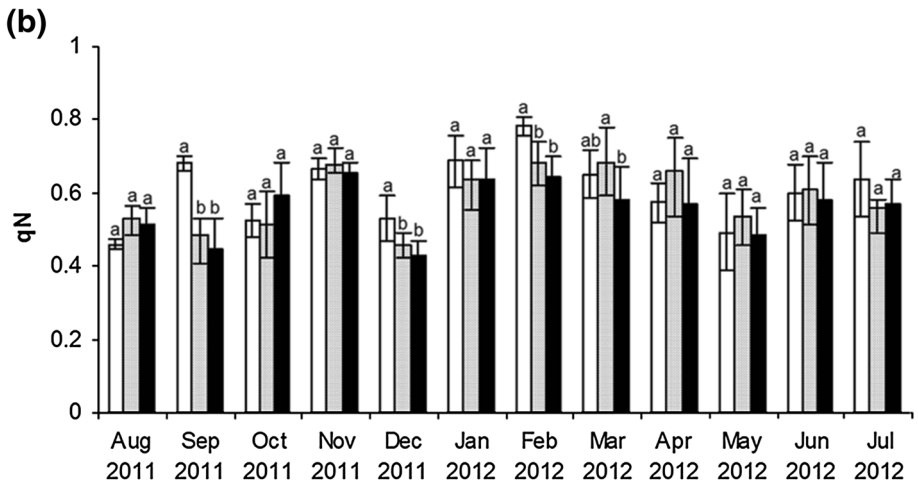




**Fig. 10** Comparison of **a** photochemical quenching (qP) and **b** non-photochemical quenching (qN) for leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) conditions. Values are mean ± SD ($n = 3$–4 plants). Means in the same month, followed by *different letters* indicated significant differences between shade regimes ($P < 0.05$; Tukey HSD's test)

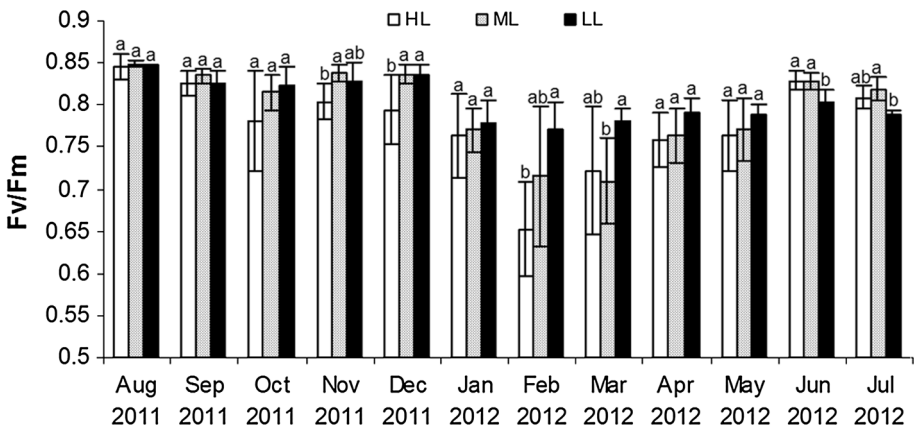



**Fig. 11** Comparison of $F_v/F_m$ ratio for leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50 % shade (ML), and 80 % shade (LL) conditions. Values are mean ± SD ($n = 3$–4 plants). Means in the same month, followed by *different letters* indicated significant differences between shade regimes ($P < 0.05$; Tukey HSD's test)

the energy absorbed by light-harvesting complexes (Bajkan et al. 2012), the absorbed light energy not utilized in photochemistry is often dissipated thermally (Martin et al. 2010). Furthermore, too high heat dissipation level might cause "chlorotic" at leaves. It was similar with phenomena of the lowest SPAD value of HL leaves on February–March 2012 (Fig. 3).

The regular value 0.75–0.85 of $F_v/F_m$ ratios have been considered normal for unstressed plants (Hunt 2003), and decline of $F_v/F_m$ under 0.75 could indicate a disturbance in or damage to the photosynthetic apparatus that due to photoinhibition (Litchtenthaler et al. 2005). HL and ML got photoinhibition on February and March 2012 (Fig. 11), probably was caused mainly by low

temperature. Photosynthesis is inhibited by low temperature, in part as an impact of reversible or reversible damage to photosynthetic structures (Robakowski 2005). The combination of low temperature and high light may affect leaf membranes and destruct the photosynthetic apparatus of higher plants (Krause 1994). Furthermore, chronic photoinhibition of HL and ML leaves might cause decoloring of photosynthetic pigments such as chlorophyll and carotenoids (Powles 1984; Takahashi et al. 2002).

In contrast with some studies, where photoinhibition was reported upon exposing shade-adapted plants to high light stress (Khan et al. 2000; Xu et al. 2009), we found that LL plants sustained low susceptibility for photoinhibition. In the present study, although $F_v/F_m$ of LL leaves declined during cold months, the values were always higher than 0.75 (Fig. 11) and never showed chronic photoinhibition level. LL seedlings might have the ability to maintain photosynthetic even at low, but non-freezing temperatures because of their protection mechanisms. The response of plants grown in darkness to low temperature had little effect on the PSII complex compared with those under light (Alves et al. 2002). Although the mechanism is not clear, we suggested that LL had a mitigation strategy of the leaf to absorb incident radiation and, therefore, decrease the quantity of excess excitation energy that has to be dissipated. This result agrees with those of Pompelli et al. (2010) and Huang et al. (2011) who also found no photoinhibition in plants grown under shade.

Acclimation to various light intensities may have an influence not only on photosynthesis, but also on several physiological and biochemical processes, which are not directly related to photosynthesis. Gray et al. (1997) reported that light as the fundamental energy source for all photoautotroph's affected PSII excitation pressure to extend beyond photosynthetic acclimation, by influencing the expression of a nuclear gene involved in low temperature acclimation. Furthermore, the expression levels of several photosynthesis- and hormone-related genes were significantly affected by the light intensity (Majláth et al. 2012). Currently, we are investigating the protein expressions in *R. mucronata* leaves under shade regimes by a proteomic approach.

## Conclusions

The results confirm that the seasonal change of photosynthetic capacity was affected strongly by carboxylation efficiency. The photosynthetic performance of *R. mucronata* seedlings under shade regimes, however, could not be attributed to variability in chlorophyll, $C_i$, ΦPSII, ETR or qP values but more to differences in carboxylation efficiency, $g_{max}$, and $E_{max}$, respectively. HL and ML plants had higher $P_N$, $g_s$ and $E$ than the LL ones. Nevertheless, LL leaves sustained low susceptibility to photoinhibition. Our findings indicate that seedling grown under moderate shade condition showed better ability to maintain a high carbon fixation capacity. This result is important to elucidate the zonation pattern of mangrove and also to clarify the suitable shading level during nurse phase of *R. mucronata* upon reforestation and cultivation.

**Author contribution** A. Nose designed and supervised the whole research work. T. Z. Ulqodry and F. Matsumoto conducted the experiment, analyzed data and wrote the manuscript draft. Y. Okimoto and S. H. Zheng corrected some parts of the manuscript.

**Acknowledgments** This work was supported by The Rendai-Student Supporting Program 2011-2012, United Graduate School of Agricultural Sciences, Kagoshima University, Japan. The authors are grateful to Directorate General of Higher Education (DIKTI), Republic of Indonesia for the PhD Grant of T. Z. Ulqodry.

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