# Studies on Mangrove Photosynthetic Performances in Relationship with Zonation and Productivity

**(光合成特性からみたマングローブ林の帯状構造と生産特性に関する研究)**

**TENGKU ZIA ULQODRY**

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## DOCTOR DISSERTATION

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the Degree of Doctor of Agriculture

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**Abbreviations.**

| | |
|---|---|
| $C_i$ | Intercellular $CO_2$ concentration |
| E | Transpiration rate |
| $E_{max}$ | Maximum transpiration rate |
| ETR | Electron transport rate |
| Fv/Fm | Ratio of variable to maximum chlorophyll fluorescence |
| HEPES | Hydroxyethylpiperazine N-2 ethane sulphonic acid |
| $g_{max}$ | Maximum stomatal conductance |
| $g_s$ | Stomatal conductance |
| PAR | photosynthetically active radiation |
| $P_{max}$ | maximum photosynthetic rate |
| $P_N$ | Net photosynthetic rate |
| PQ | Photosynthetic quotient |
| PSII | Photosystem II |
| qN | Non-photochemical quenching |
| qP | Photochemical quenching |
| SPAD | Soil Plant Analysis Development |
| Vpdl | Vapour pressure deficit between the leaf and air |
| ΦPSII | Quantum yield of Photosystem II |

# CHAPTER 1

## General introduction

Mangrove is an important and unique coastal ecosystem in the tropic and subtropic area which grow at the interface between land and sea where may be no other group of plants with such highly developed morphological and physiological adaptations to extreme conditions. Mangroves play an important function because of their specific habitat in intertidal zone (McLeod and Salm, 2006), high productivity (Clough, 1998; Okimoto *et al*., 2008), and their specific species zonation (Bunt, 1996; Youssef and Saenger, 1999).

Mangrove habitats are generally restricted to the intertidal zone, which is the strip of coast starting from the lowest low water level up to the highest high water level (Giesen *et al*., 2007). Mangroves are not just transitional in nature, having some elements of terrestrial and marine ecosystems, but also having ecological characteristics all their own (Alongi, 2009). Subjects to daily, monthly, and annual variations in their physical habitat, mangroves have a remarkable ability to survive with stress conditions (McLeod and Salm 2006). Mangroves are highly adapted to the coastal environment, with exposed breathing roots, extensive support roots and buttresses, salt-excreting leaves, and viviparous water dispersed propagules (Kathiresan and Bingham, 2001). Physiological adaptation is one reason why mangroves are so successful across the intertidal seascape (Alongi, 2009).

Light, salinity and flooding are considered as the important factors in mangrove habitat. Mangrove seedling light responses are important for mangrove forest

dynamics (Krauss *et al*., 2008). Mangrove plants have abilities to tolerate or avoid the anoxia and salt conditions due to saline-waterlogging process. Some responses to saline-waterlogging conditions include the stomatal closure, rapid leaf senescence and shedding, increased foliar sodium, reduction in water uptake and transpiration, and formation of adventitious roots (Alongi, 2009).

Living in specific habitat at land-sea boundary area (Fig 1.1), mangroves are excellent community to test the interaction of "resource" and "regulator" gradients to explain adaptation pattern in intertidal zone. Gradient in light and soil fertility are representative resources, and salinity and flooding hydroperiod are regulators of mangrove physiological response (Cardona-Olarte *et al*., 2006). Explaining potential eco-physiological responses of mangroves to light, salinity, and flooding were important contributions of mangrove reviews (Ball, 1986, 2002; Smith *et al*., 1989; Popp *et al*., 1993) and are still need being tested and developed by contemporary science programs (Krauss *et al*., 2008).

Mangroves are not only a transition habitat from the land to marine ecosystems, but also have a higher carbon fixation capacity than terrestrial forests (Lugo and Snedaker, 1974; Donato *et al*., 2011; Okimoto *et al*., 2013). Mangroves are among the most carbon-rich forests in the tropics, containing on average 1,023 ton carbon per hectare (Donato *et al*., 2011). In the coastal ecosystem, mangrove together with salt marshes and sea grasses are referred to as the earth's 'blue carbon sinks', which capture and store between 235 and 450 trillion tons of carbon every year (Nellemann *et al*., 2009; Okimoto *et al*., 2013). For this reason, mangroves have been considered as an important carbon sink in coastal ecosystems (Ong 1993). The higher carbon

fixation capacity of mangrove trees shows possibilities for their use in Clean Development Mechanism (CDM) programs of the Kyoto Protocol (Okimoto *et al*., 2007). Planting mangroves could offer the potential to sequester carbon and tap into this carbon market. The CDM assists in the reducing greenhouse gas emissions into the atmosphere by establishing a market where governments can pay for carbon emission reductions. Afforestation and reforestation (A/R) mechanisms remove carbon dioxide from the atmosphere and store it in carbon pools through the photosynthesis of the planted trees.

Like other forests, mangrove stands vary in size and age over time, and therefore vary in rates of production and in the balance between photosynthesis and respiration. Seasonal and annual changes in solar radiation, temperature, rainfall, evapotranspiration, daylength, or other factors, such as time lags, all play roughly equal roles in affecting mangrove primary production. The ability to estimate the role of mangroves in regional and global carbon cycling is an accurate estimation of net primary production (Alongi, 2009). Okimoto *et al*. (2013) point out that a critical important function of mangrove forest is to sequester and accumulate great amounts of the greenhouse gas $CO_2$, which is of primary significance in attempts to address the effects of global climate change. Afforestation and reforestation (AR)-Clean Development Mechanism is one of the prime countermeasures and the process of reducing emissions from deforestation in developing countries, incorporating conservation, sustainable management and enhancement of forest carbon stocks has been one of the most controversial issues in the climate change debate.

The other unique and interesting point from mangroves are not only their high productivity and adaptation ability in intertidal zone, but also their zonation pattern. Vegetational zonation of the mangroves, a frequently conspicuous feature, has long attracted scientific interest (Bunt, 1996). These zonation patterns are generally well correlated with the frequency and duration of tidal immersion. Tidal characteristics exert their influence on mangrove vegetation through intermediate factors, which either directly affect growth or are resources required for growth. Such factors include the degree of soil saturation, the form and availability of nutrients and the salinity of surface and soil water (Ball, 1988).

Different mangrove species tend to occupy specific zone or specific habitat zonation (Bunt, 1996; Youssef and Saenger, 1999). Species differences in mangrove responses to the interactive effects of some stress conditions might explain important differences in mangrove forest structure (Krauss *et al*., 2008). In Southeast Asia mangroves generally occur in five zones (Giesen *et al*., 2007): (i) one on the highly exposed seaward side that is inundated during all high tides; (ii) one on less dynamic, exposed, seaward sides, inundated by all high tides; (iii) a central, well-developed mangrove inundated by normal high tides; (iv) a landward/freshwater-influenced zone (the back-, hind- or rearmangrove) inundated by spring tides, and (v) a zone occurring along brackish to almost fresh streams and/or occasionally inundated by exceptionally high tides.

In the west Indonesia, White *et al*. (1989) and Whitten *et al*. (2000) identified that mangrove showed a characteristic zonation as characteristic: (1) *Avicennia,* the mangrove pioneer species, growth commonly in low intertidal swamps; (2)

*Rhizophora*, occupy dominantly in intermediate zone at the mid-tidal level; and (3) *Bruguiera*, establish commonly on backside land area (Fig 1.2). Based upon inferences made from intertidal distributions, these three species appear to differ in their sensitivity to salinity and flooding, tissue water potential and ion concentration (Naidoo, 1985).

Photosynthesis processes are among the most sensitive indicators of environmental stresses, conducive to use in monitoring environmental conditions (Ball, 1986) and also important aspect to elucidate plant productivity. Another consideration relevant to early growth of mangroves is that its photosynthetic performance is dependent upon many aspects (Krauss *et al*., 2008). Some previous studies have investigated effects of habitat conditions on mangrove seedlings growth (Kathiresan and Bingham 2001; Saenger 2002; Naidoo, 2006; He *et al*., 2007), but there are limited information available for mangrove photosynthetic responses (Krauss *et al*., 2006; Cardona-Olarte and Twiley, 2006). Studies of photosynthesis in different species may give some indication of how differences in photosynthesis capacity and sensitivity to environmental conditions relate to mangrove performance under field conditions. Nevertheless, Ball (1986) point out that the role of photosynthesis in the growth of individual mangrove, interspesific competition, and the productivity cannot be over-emphasized. Understanding mangrove photosynthesis performance is fundamental to understanding long-term dynamic of mangrove forests.

Many articles describing plant photosynthetic performance to various abiotic conditions have been published over the last decade, but only a handful have targeted mangrove species (Ball and Critchley, 1982; Björkman *et al*., 1988; Kawamitsu *et al*.,

2003; Krauss *et al*., 2008). Mangroves might exhibit distinctions in photosynthetic capacity and sensitivity to environmental conditions for different species (Ball, 1986). Basak *et al.* (1996) found that significant intra- and interspecific variation in photosynthetic activity from mangrove species, suggesting that the rates of photosynthesis may have an underlying genetic basis.

Abiotic stress-induced changes in the relative rates of photosynthesis, in turn, influence overall growth rates of mangrove. Mangrove posses the special ability to cope with a wide range of shading, salinities and flooding conditions. The studies of photosynthesis in relation to shading, salinity and flooding conditions will useful to identify the functional interactions of shading, salt and flooding tolerant properties to crop plants and also for mangrove restoration.

Mangroves show characteristic $C_3$ photosynthesis (Ball, 1986; Kathiresan, 2001; Kawamitsu *et al*., 2003[a]). There is no convincing evidence in mangroves of environmentally induced shifts from $C_3$ to either $C_4$ or CAM photosynthetic biochemistry (Ball, 1986). Although belongs to the $C_3$ photosynthesis plants, mangrove also can be classified as "seaweed", since it can grow in submerged and high salinity conditions, whereas $C_3$ plants could not survive (Kawamitsu *et al*., 2003[a]). Photosynthetic rates of mangrove leaves under aqueous conditions can be evaluated by the measurement of $O_2$ evolution with a liquid-phase $O_2$ electrode, which is stoichiometrically equivalent to fixed $CO_2$ (Delieu and Walker, 1981; Pimentel, 1999). This approach justified that the ratio of $O_2$ evolution to $CO_2$ fixation is 1:1 (Espie, 1986). In other side, some process occurred in photorespiration, Calvin cycle, and photosystem II contribute in evolution or absorption of both $O_2$ and $CO_2$

during $C_3$ plant photosynthesis. For example, Ribulose-1,5-biphosphate carboxylase (Rubisco) is the primary $CO_2$-fixing enzyme for photosynthetic carbon reduction in leaves and it requires $CO_2$ as a substrate, but it also fixes $O_2$ and is thus bifunctional (Kawamitsu and Boyer, 2003). Therefore, the ability to directly determine the $O_2$ evolution and $CO_2$ uptake rate of leaf samples under liquid phase simultaneously and monitor how the rates change in response to stimuli will create deep implications in furthering the understanding of a wide array from single leaf to complex ecosystems (Strovas *et al*., 2010).

The ability to monitor $O_2$ production and $CO_2$ uptake levels simultaneously during photosynthetic rate under aqueous conditions was limited and no information for mangrove leaves. Simultaneous measurement of $CO_2$ and $O_2$ also can be measured with a gas chromatograph or mass spectrometer (MS) but is very expensive and requires long time to complete an analysis of a sample (Sipior *et al*., 1996). With the advent of a new type of optical electrodes, the so-called opt(r)odes, This study try to improve the simultaneous measurement of $O_2$ evolution and $CO_2$ uptake under aqueous conditions.

Leaf photosynthetic $O_2$ evolution and $CO_2$ uptake are fundamental mechanisms that support oxygen and carbon ecosystems from the individual plant to the global scale. Almost all of the published work on the photosynthetic responses on mangroves have been conducted mainly either $O_2$ evolution or $CO_2$ uptake independently (Ball and Critchley, 1982; Okimoto *et al*., 2007). Until now, there is no study about the mangrove photosynthetic $O_2$ evolution and $CO_2$ uptake simultaneously. A simultaneous measurement of $O_2$ evolution and $CO_2$ uptake during

photosynthesis is essential in order to calculate the photosynthetic quotient (PQ), which is described as the molar ratio of the rate of $O_2$ production to the rate of $CO_2$ utilization (Williams and Robertson 1991).

The PQ value provide fundamental information on metabolic pathways (Taddei *et al*., 2008), balanced growth (Davies *et al*., 2003) and useful to clarify the primary productivity in an ecosystem (Lee and Bong, 2006). Some ecosystem productivity studies have been made with the assumption that PQ=1 (Nielsen and Nielsen, 2006; Suzumura *et al*., 2002) without attempting an experiment verification of this value, which could affect data interpretation for tropic balance (Taddei *et al*., 2008). Until now, there is no detail information about PQ values of mangrove leaves.

The main objective of this study is to investigate photosynthetic performance in mangrove leaves as regards their productivity and adaptability mechanisms. This information will hopefully be useful not only to explain productivity but also to elucidate the mangrove distributional patterns, or ‘‘zonation’’. Furthermore, understanding potential photosynthetic performances of mangroves to light, salinity, and flooding were important contributions for diagnosing successful mangrove within tropic intertidal zone, managed or natural. An accurate and reliable estimation of the net $CO_2$ fixation capacity of mangrove forests from different zonation become necessity to apply mangrove productivity for a CDM project that achieve reductions of greenhouse $CO_2$ (Okimoto *et al*., 2007). The using of mangrove photosynthetic approach to elucidate mangrove productivity and zonation, will be key factor on mangrove rehabilitation.

The environmental, social and economic impacts associated with the decline and degradation of mangrove forest ecosystems have been recognised and have led to a greater appreciation of their importance and value. Rural communities in coastal areas depend on mangroves as their primary source of income generation, firewood, charcoal, medicine and other basic necessities such as timber for housing (Wekesa and Aswani, 2015). In the last few decades, mangroves have started to be replanted and contributes to rehabilitate the coastal ecosystems. However, almost of the planted areas of mangroves in the Southeast Asia are abandoned due to lack of any forest management. Wise management of these resource is therefore essential for the sustainable use and also for the socio-economic welfare of the coastal inhabitants (Wekesa and Aswani, 2015). The CDM approach could lead to multidimensional benefits that would seek to enhance ecosystem resilience, conserve biodiversity, protect vulnerable communities, and contribute to rural development (Mustafa and Shapawi, 2015). The successful of mangrove reforestation activity in coastal rural development will keep the main function of mangrove as carbon pool simultaneously.

As noted earlier, photosynthesis may vary with many factors, especially light intensity, salinity, flooding, and species composition (Alongi, 2009). The first study investigated the seasonal photosynthetic responses and chlorophyll fluorescence in mangrove seedlings under shade regimes. By studying the mangrove photosynthetic performance under shade regimes, it will clarify the most suitable shading level during nurse phase of mangrove upon reforestation and cultivation. In the second study, the improved method of simultaneous determination of photosynthetic $O_2$ evolution and $CO_2$ uptake of mangrove leaves under aqueous conditions will be discussed. The important result from simultaneous measurement of $O_2$ evolution and

$CO_2$ uptake is the ability to explore the photosynthetic quotient (PQ) values of mangrove leaves under aqueous conditions as shown in third study. The result in third study was important because will elucidate the zonation pattern of mangrove based on photosynthetic response from each mangrove species. In the General Discussion, the photosynthetic characteristics of mangrove to light, salinity and flooding conditions will be discussed and summarized. Generally, the studies on mangrove photosynthetic performance and its characteristics based mainly on physiological mechanism. In addition, this study propose for using biochemical and protein expression approach of the combined stress to clarify mangrove zonation and to develop the appropriate strategies to sustain mangrove production.

**Fig 1.1.** Mangroves in their natural habitat, Banyuasin Peninsular, Sumatera, West Indonesia.

**(a)** ***Bruguiera gymnorrhiza***

**(b)** ***Rhizophora mucronata***

**(c)** ***Avicennia marina***

**Fig 1.2.** Three mangrove species from different mangrove zonation are found commonly in the coastal area of West Indonesia which grown in green house. They are (a) *Bruguiera gymnorrhiza*, establish commonly on backside land area; (b) *Rhizophora mucronata*, occupy dominantly in intermediate zone; and (c) *Avicennia marina*, the mangrove pioneer species, growth commonly in low intertidal swamps.

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# CHAPTER 2

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

### 1. Introduction

*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-Speer *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. *R. mucronata* had a role as main plant in the reforestation thinned site in tropical coastal area (Srivastava *et al*., 1988) and produced more leaf litter than the reforestation unthinned and natural sites (Wang'ondu and Virginia, 2010).

While thinning activity contribute 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.

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 Crithcley, 1982). On the other hand, *Bruguiera sexangula* responded favourably to sunlight at low light level and is considered as relatively shade tolerant species (Krauss and Allen, 2003).

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. Temperatur 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 $^0$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, this study also investigated the photosynthetic responses of sunned

and shaded leaves of *R. mucronata* seedling for 1 year, while the temperature was different at each months.

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 in order to estimate mangrove productivity with photosynthetic growth model (Okimoto *et al*., 2008).

## 2. 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 five 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 midday at July 20, 2012, a sunny cloudless day, and showed that the actual photosynthetically active radiation (PAR) was 1728, 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 from each

day were determined, and these daily values were averaged over a month to get the data points displayed in Fig 2.1.

**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 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 in sunny days from the morning (08:00 h, local time) until close to mid-day (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 1000 to 0 μmol $m^{-2}$ $s^{-1}$ (1000, 500, 250, 100, 50, 0 μmol $m^{-2}$ $s^{-1}$), and leaf temperature was controlled at 30 $^0$C, vapour 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 $P_N$ was measured from 20 to 38 $^0$C under PAR, VpdL and $CO_2$ input were 1000 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. The quantifying of $P_N$ as $C_i$ function was done by changing the $CO_2$ concentration at the leaf surface from 0 to 1000 μmol $mol^{-1}$, PAR 1000 μmol $m^{-2}$ $s^{-1}$ and leaf temperature 30 $^0$C.

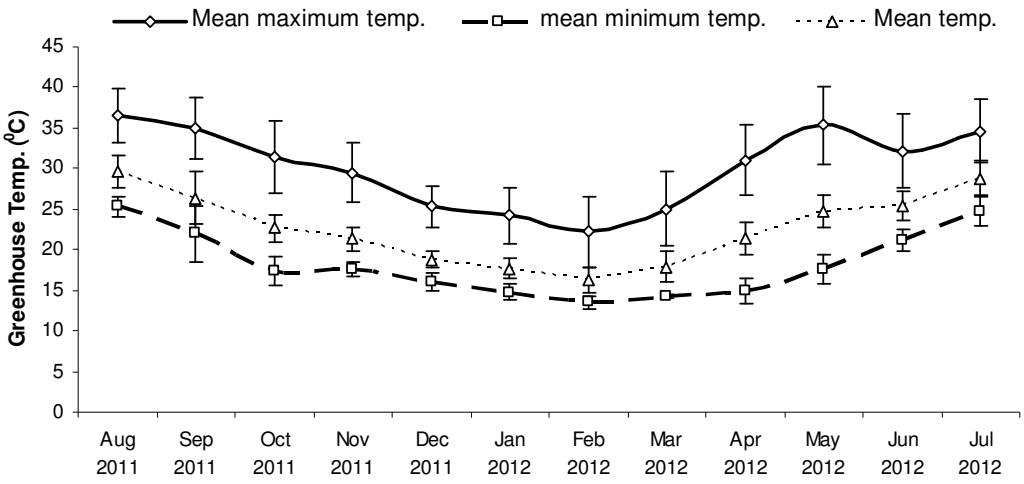


**Fig. 2.1** Mean monthly, mean monthly minimum, and mean monthly maximum of greenhouse air temperature during 1 year experiment. Value is mean $\pm$ SD (n=number of days in each months). Especially during cold months (December 2011-March 2012), the minimum greenhouse temperature was arranged more than 10 $^{0}$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 minutes dark adaptation using a leaf clip. Minimum fluorescence (Fo) was determined by a weak red light and maximum fluorescence (Fm) was induced by a 0.8 s pulse of 2000 μmol $m^{-2}$ $s^{-1}$ PAR. The steady state fluorescence (Fs) was recorded and a second saturating pulse was applied to determine the maximum light-adapted fluorescence (Fm'). 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 (Fo') 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, ΦPSII = (Fm'-Fs)/Fm'; maximum quantum efficiency of fluorescence PSII, Fv/Fm = (Fm-Fo)/Fm; photochemical quenching coefficient, qP = (Fm'-Fs) / (Fm'-Fo'); non-photochemical quenching, qN = (Fm-Fm') / (Fm-Fo'); and electron transport rate, ETR = ΦPSII x PAR x 0.5 x 0.84. PAR corresponds to the flux density of incident photosynthetically active radiation, 0.5 was as a factor that accounts for the portioning of energy between PSII and PSI, and 0.84 was assumed from an average of 84% of the incident light were absorbed by the leaf.

**Soil plant analysis development (SPAD) measurement**

SPAD 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 differences between means. Significant differences are reported as $P < 0.05$.

## 3. Results

### Leaf morphology and SPAD value.

Shade treatments affected *R. mucronata* leaf morphology. LL leaves were larger than HL and ML leaves. Leaf colour of LL-plants were dark green, while those of ML- and HL-plants were green and light green, respectively (Fig 2.2).

SPAD readings being in tight correlation with chlorophyll content (Markwell *et al*. 1995) showed similar HL<ML<LL pattern for each months (Fig 2.3). 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 Ci.

Variation of $P_N$ responses to light intensity at 30 $^0$C of leaf temperature showed almost similar trends for all three treatments, increased simultaneously with PAR escalation until reaching their saturation point (Fig 2.4).

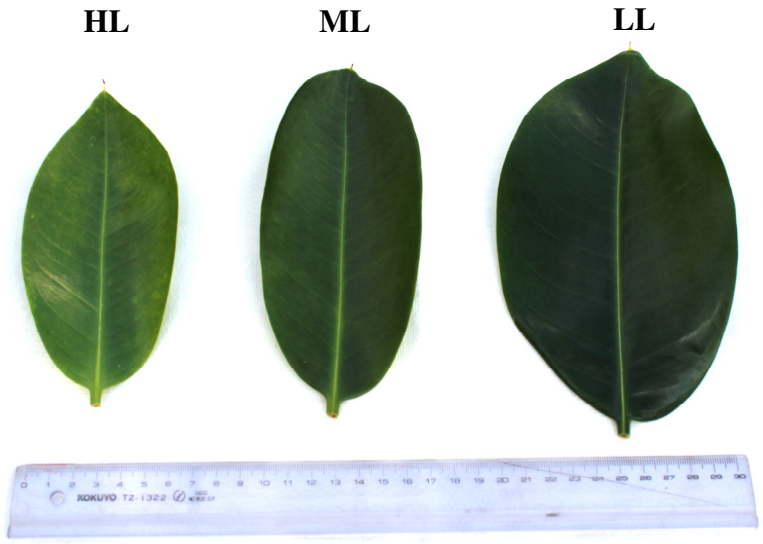


**Fig. 2.2** Leaves of *R. mucronata* from the various shade treatments, full sunlight (HL), 50% shade (ML), and 80% shade (LL). They were collected on September 16, 2012.

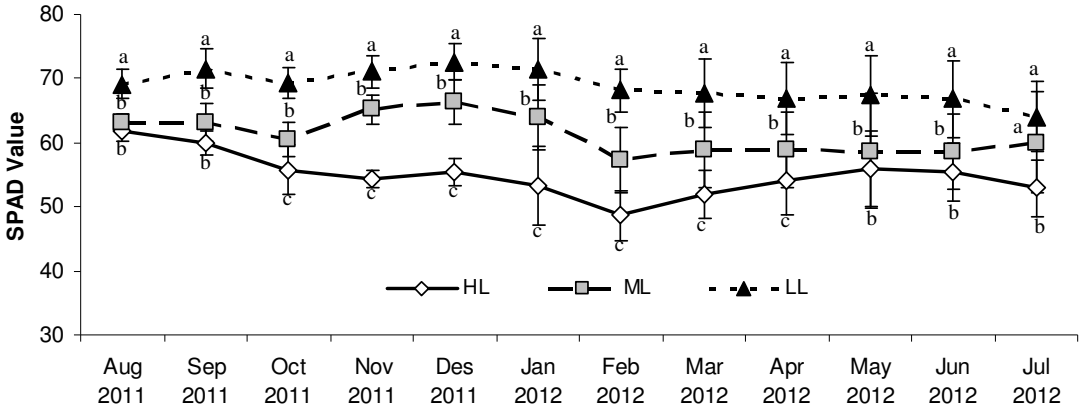


**Fig. 2.3** SPAD value in leaves of *R. mucronata* grown under full sunlight (HL), 50% shade (ML), and 80% shade (LL) conditions. Value is mean ± SD (n=3-4 plants). Mean in the same month, followed by different letters indicated significant differences between shade regimes ($P<0.05$; Tukey HSD's test)

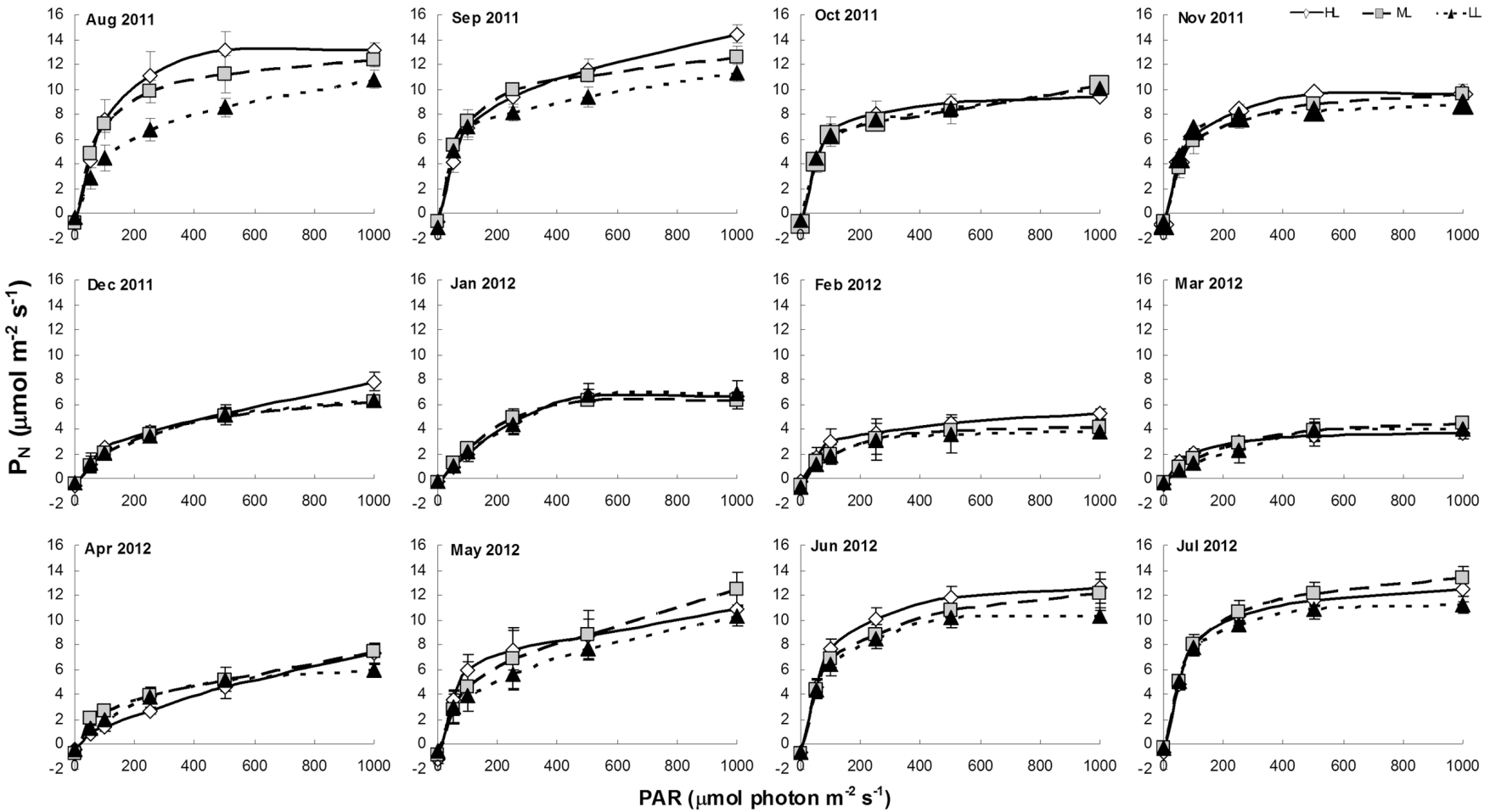


**Fig. 2.4** Response of net photosynthetic rate ($P_N$) to increasing photosynthetically active radiation (PAR) in the leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. They were measure at leaves temperature 30 $^0$C. Value is mean $\pm$ SD (n=3-4 plants).

The light responses of $P_N$, $g_s$ and E were determined using the rectangular hyperbola model (Okimoto *et al*. 2008; Table 2.1):

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

where P is $P_N$ of individual leaves at light intensity of *I* (μmol photons $m^{-2}$ $s^{-1}$), then α and β are coefficients to determine the convexity of the hyperbola. When used to model of conductance and transpiration responses, P was substituted to represent the $g_s$ and E values in Eq.1. HL and ML had higher $P_N$, $g_s$ and E than LL leaves while PAR increasing.

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 2.1). The light saturation points of all treatments were commonly at PAR level around 1000 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. In 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 2.4).

**Table 2.1** The values of $P_{max}$, $g_{s\text{-}max}$, and $E_{max}$ at saturating level of PAR 1000 µmol photons $m^{-2}$ $s^{-1}$ and leaf temperature 30 $^0$C in leaves of *R. mucronata* grown under full sunlight (HL), 50% shade (ML), and 80% shade (LL) conditions. 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.

| Code | Month | Equation | | | $P_{max}$ | $g_{s\text{-}max}$ | $E_{max}$ |
|---|---|---|---|---|---|---|---|
| | | $P_N$ | $g_s$ | 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+0.34 I) | 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) | gs=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) | gs=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) | $g_s$=I/82.27+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 |

Under light saturation, $P_{max}$ showed a positive correlation with $g_{max}$ and $E_{max}$ (Fig 2.5). 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 $P_{max}$. In this study, it was found that although the highest value of $g_{max}$ and $E_{max}$ of ML were lower than HL, but their highest $P_{max}$ value were tendency similar.

**Effects of temperature on photosynthesis.**

The quadratic curves were fitted to describe the temperature responses of $P_N$ (Fig 2.6). The results showed that relationship between $P_{max}$ and leaf temperature indicated a broad peak for difference season. During mid-high temperature months between August-November 2011 and May-July 2012, $P_{max}$ was obtained at leaf temperature 29-34 $^0$C, and between 23-29 $^0$C on 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 $^0$C, while ML (13.8 μmol $m^{-2}$ $s^{-1}$) ensued on July 2012 at 33 $^0$C.

$P_N$ shows seasonal variation while leaf temperature was set at 30 $^o$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 $^0$C than HL and ML leaves (Fig. 2.7).

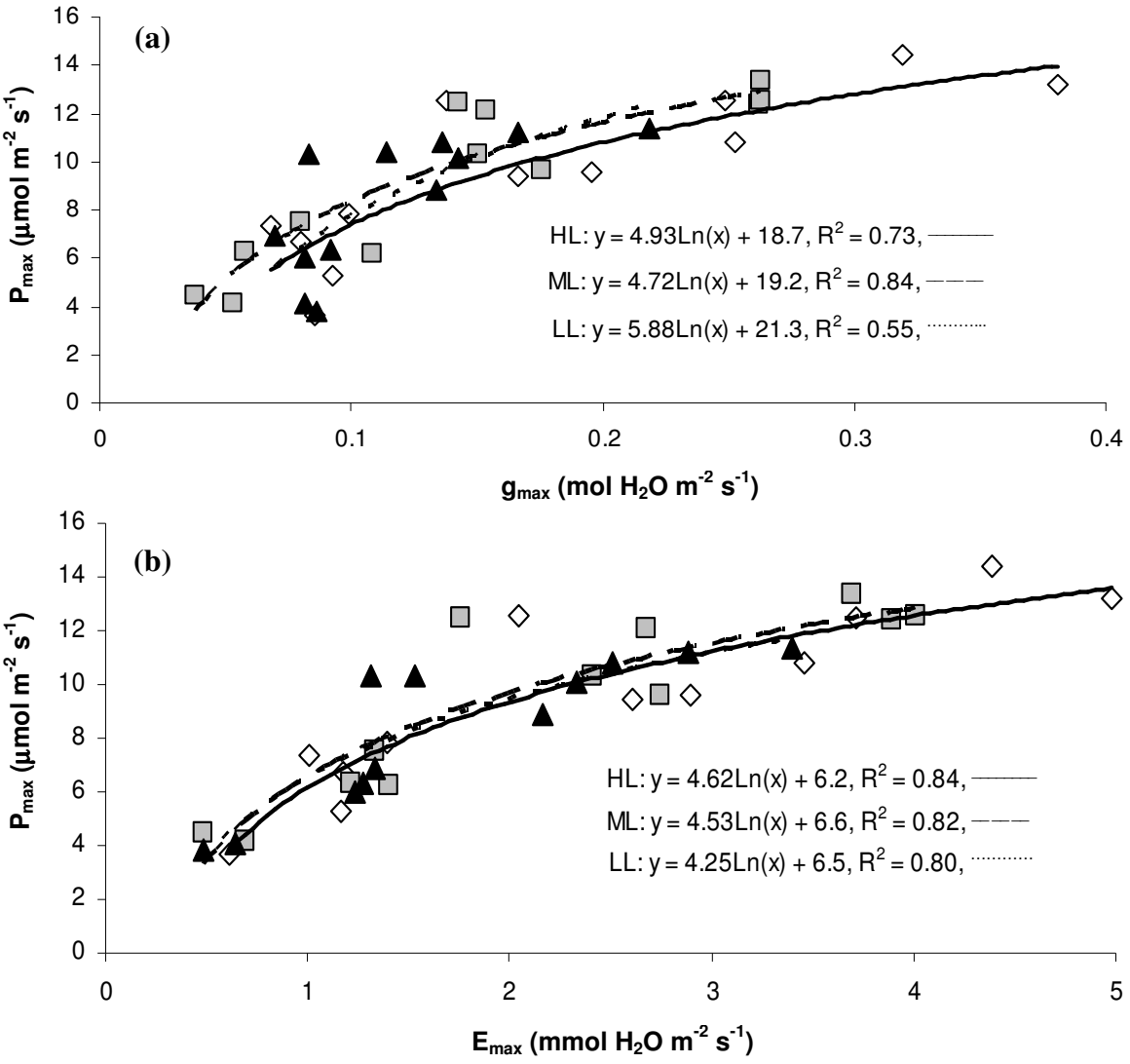


**Fig. 2.5** Maximum photosynthetic rate ($P_{max}$) as a function of (a) maximum stomatal conductance ($g_{max}$) and (b) maximum transpiration rate ($E_{max}$) for *R. mucronata* seedlings grown under full sunlight (HL, diamonds and solid lines), 50% shade (ML, squares and dash lines) and 80% shade (LL, triangles and dotted lines). Data plotted from monthly value of $P_{max}$, $G_{max}$ and $E_{max}$ at PAR 1000 µmol photon $m^{-2}$ $s^{-1}$ and leaf temperature 30 $^0$C.

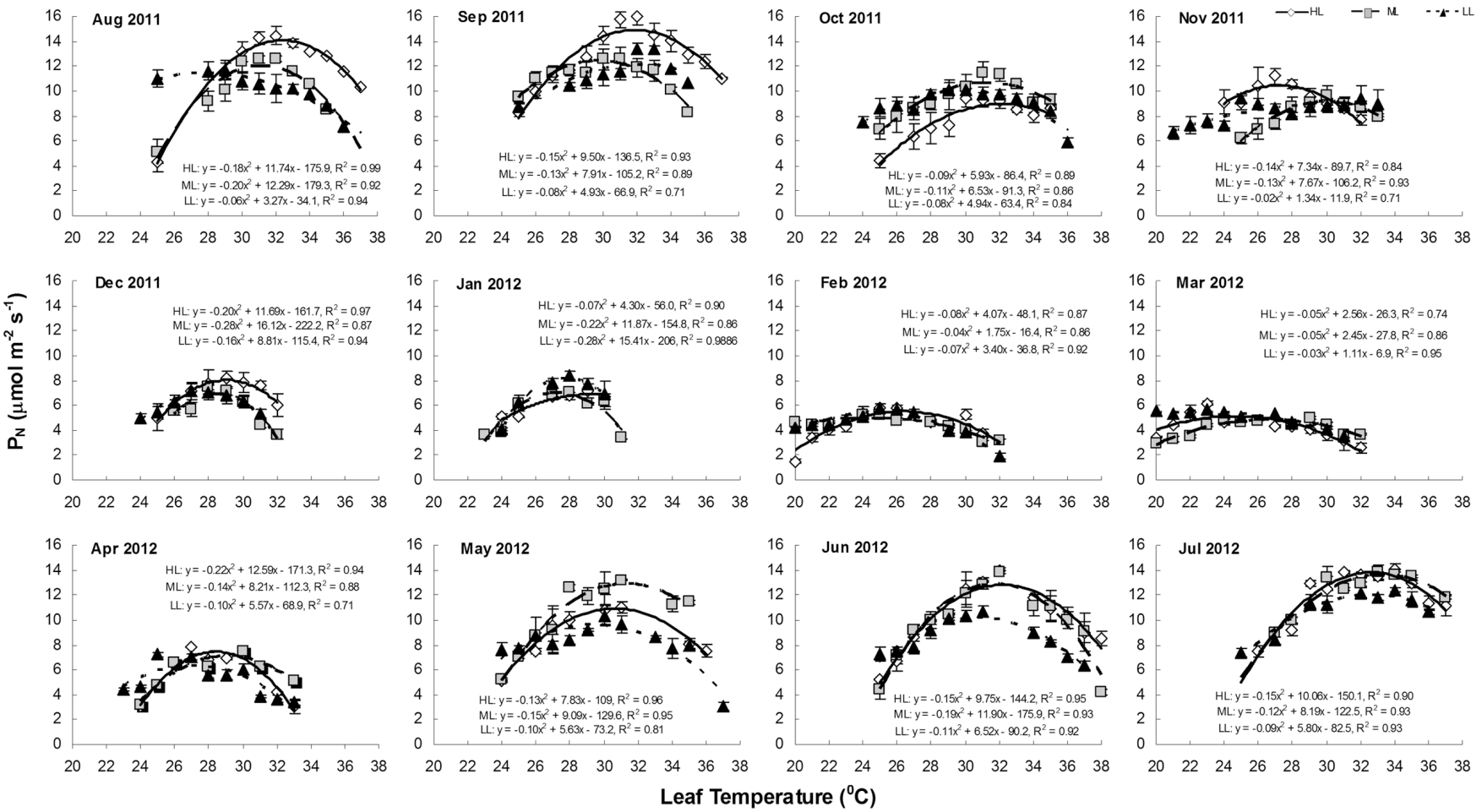


**Fig. 2.6** Response of net photosynthetic rate ($P_N$) to increasing leaf temperature *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. They were measure at PAR 1000 µmol photon $m^{-2}$ $s^{-1}$. Value is mean $\pm$ SD (n=3-4 plants)

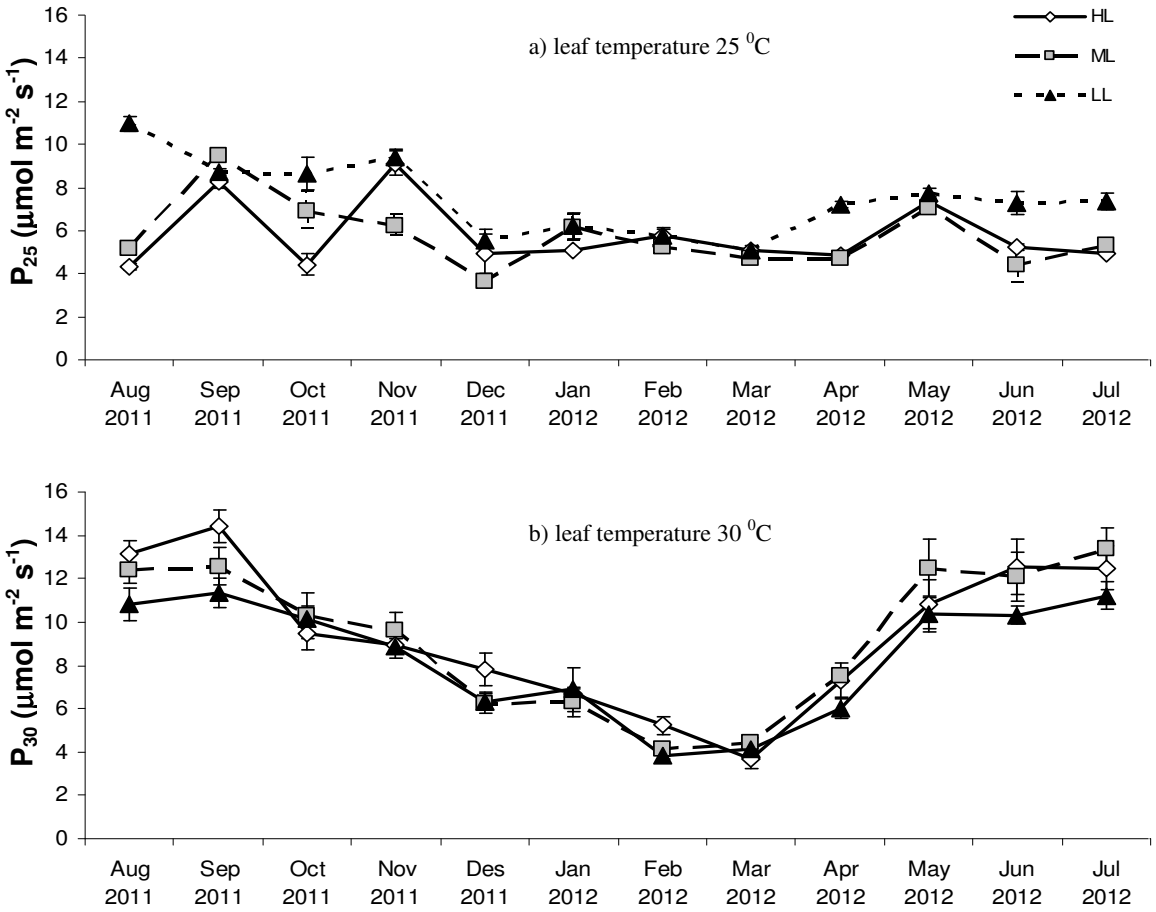


**Fig. 2.7** Net photosynthetic rate of *R. mucronata* seedlings grown under full sunlight (HL, *diamonds*), 50% shade (ML, *squares*) and 80% shade (LL, *triangles*) at (a) leaf temperature 25 $^0$C and (b) 30 $^0$C. Value is mean $\pm$ SD (n=3-4 plants)

**Effects 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 Ci tend to zero, i.e.

$$P = \frac{I}{\alpha + \beta \cdot I}$$

$$P' = \frac{[1 \cdot (\alpha + \beta \cdot I)] - (\beta \cdot I)}{(\alpha + \beta \cdot I)^2}$$

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

$$P' = \frac{\alpha}{\alpha^2}$$

$$P' = \frac{1}{\alpha} \qquad (2)$$

where P', *I* and α are initial slope 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 increasing 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 $C_i$ become infinity, i.e.

$$P = \frac{I}{\alpha + \beta \cdot I}$$

$\frac{1}{P} = \frac{\alpha}{I} + \beta$ , and *P* become $P_{max\text{-}Ci}$ while *I* become ∞

$$P_{max-Ci} = \frac{1}{\beta} \qquad (3)$$

where $P_{max\text{-}Ci}$ is the maximum photosynthetic rate response to $C_i$ and β is second coefficient determining the convexity of the hyperbola.

Figure 2.8 shows that initial slope of $P_N$ ($C_i$) had similar seasonal variation as $P_{max}$-$C_i$. Both of 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, that may indicate that temperature and light have synergic effect on the initial slopes.

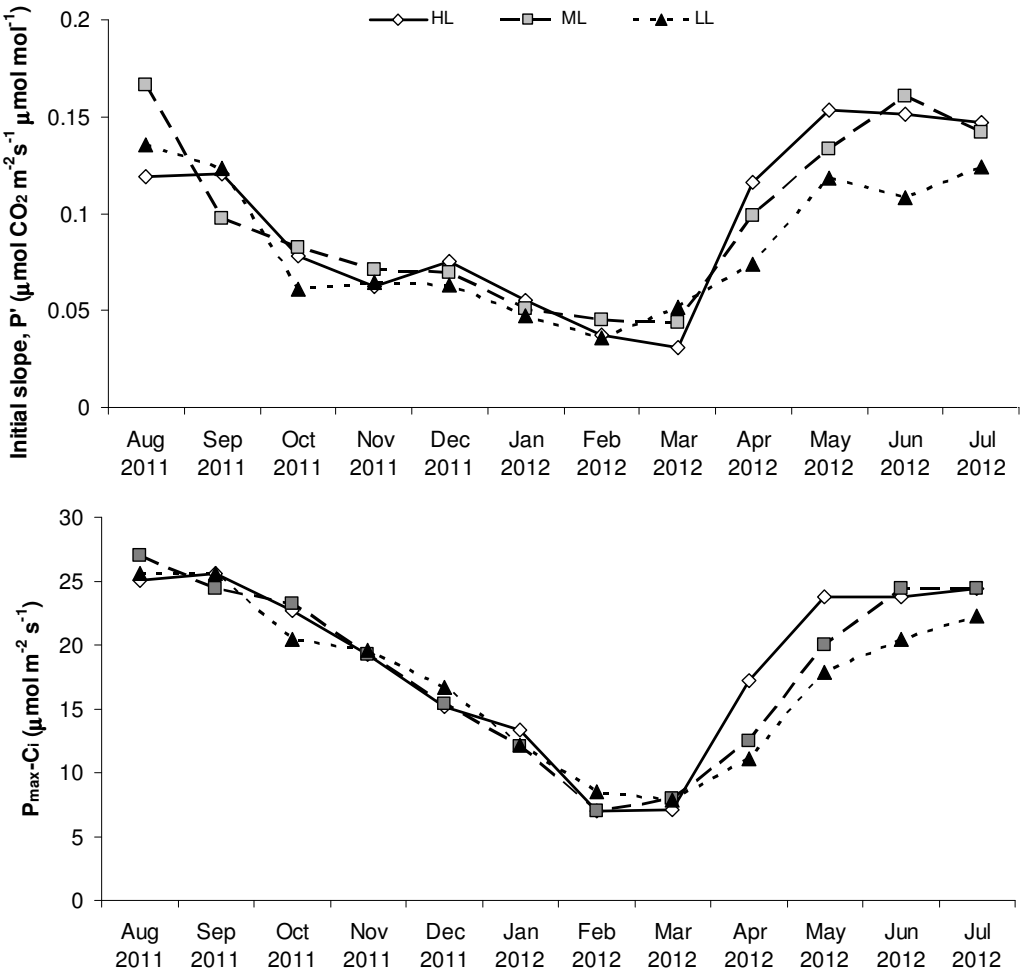


**Fig. 2.8** Monthly pattern of initial slope (P') and maximum photosynthetic rate responses to $C_i$ ($P_{max}$-$C_i$) of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL). They were measure at leaves temperature 30 $^0$C PAR 1000 µmol photon $m^{-2}$ $s^{-1}$. The values of P' and $P_{max}$-$C_i$ were calculated with Eq.2 and Eq.3, respectively.

**Chlorophyll fluorescence.**

The seasonal variation of quantum yield of PSII (ΦPSII) and electron transport rate (ETR) measured after 30 minutes 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. 2.9).

Photochemical quenching (qP) is a ratio of light energy used in the transfer of photochemical electron 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-November than in the cold months (December-March) (Fig 2.10a). Unexpectedly, the qP value for HL was high in February 2012, whereas the photosynthetic rate was low (Table 2.1). Furthermore, qN values of HL leaves exhibited a slight higher in February 2012 as compared with other months (Fig 2.10b).

A reduction in the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) can be used as an indication of photoinhibition (Björkman and Demmig 1987; Robakowski 2005). HL and ML leaves showed seasonal Fv/Fm ratio variation and exhibited a significant decreasing in February and March 2012 (Fig 2.11).

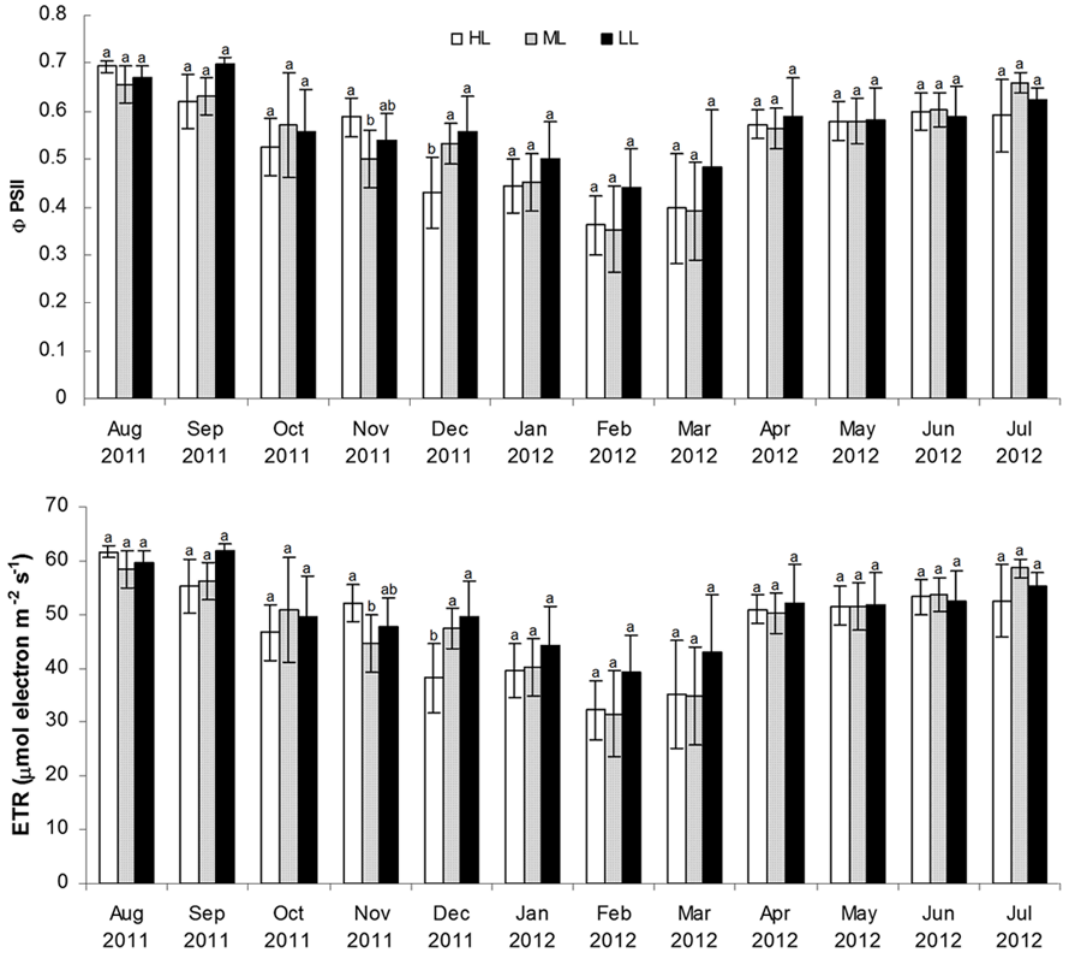


**Fig. 2.9** Quantum yield of PS II (ΦPSII) and electron transport rate (ETR) after 30 minutes-dark adaptation at leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. Value is mean ± SD (n=3-4 plants). Means in the same month, followed by different letters indicated significant differences between shade regimes ($P<0.05$; Tukey HSD's test)

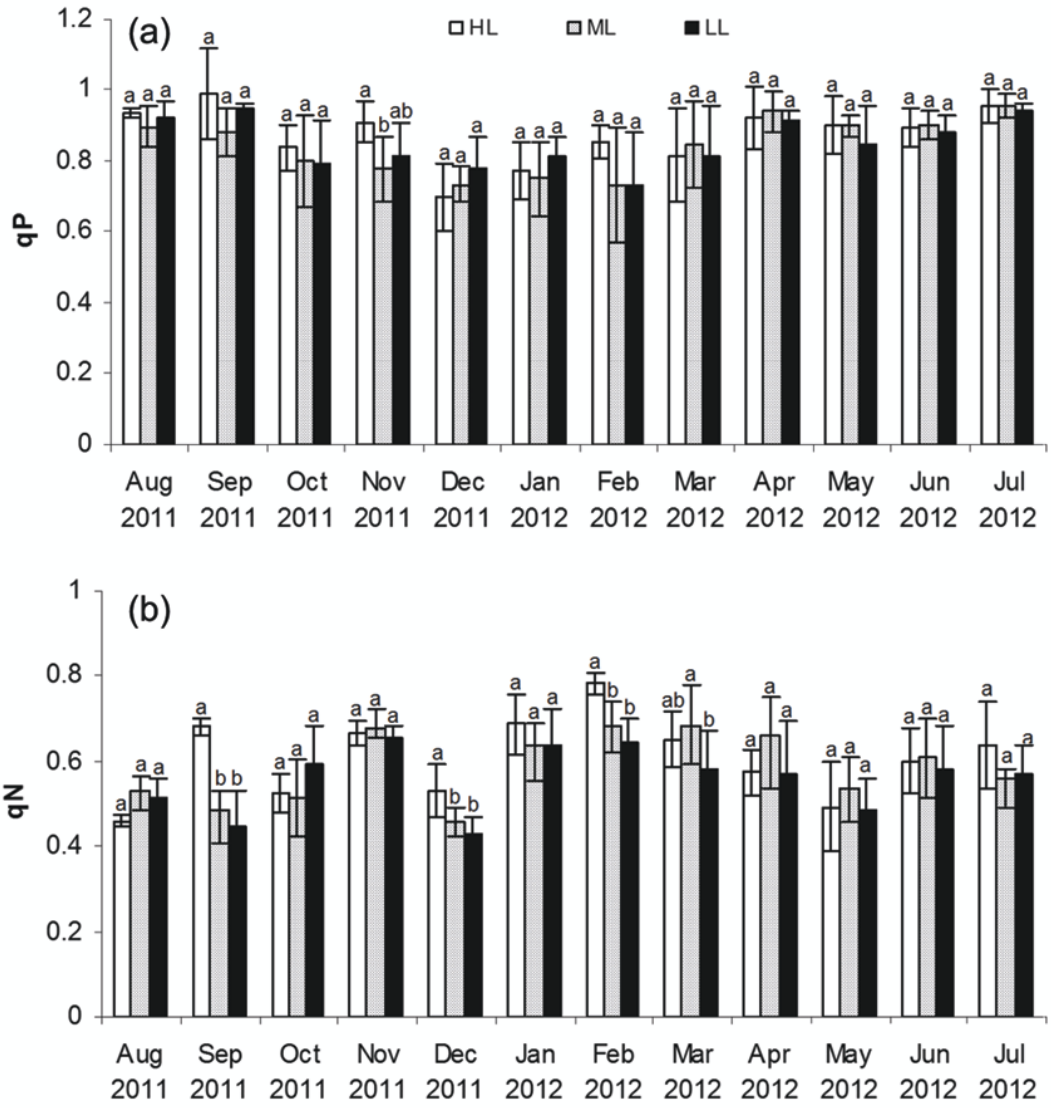


**Fig. 2.10** Comparison of (a) photochemical quenching (qP) and (b) non-photochemical quenching (qN) for leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. Value is mean $\pm$ SD (n=3-4 plants). Means in the same month, followed by different letters indicated significant dIfferences between shade regimes ($P<0.05$; Tukey HSD's test).

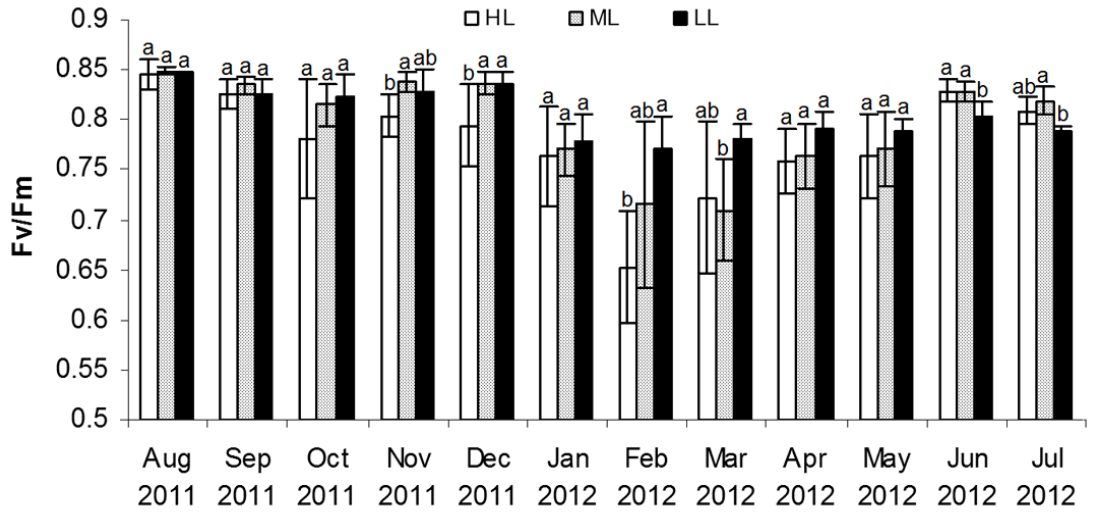


**Fig. 2.11** Comparison of Fv/Fm ratio for leaves of *R. mucronata* seedlings grown under full sunlight (HL), 50% shade (ML) and 80% shade (LL) conditions. Value is mean $\pm$ SD (n=3-4 plants). Means in the same month, followed by different letters indicated significant differences between shade regimes ($P<0.05$; Tukey HSD's test).

## 4. Discussion

The results showed significantly increased SPAD values ($P < 0.05$) and leaf sizes while in plants exposed to 50 and 80% shading (Fig 2.2 and 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 (Wittmann *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). In this study, decolouring symptom with lower SPAD value of HL and ML leaves was also found in February 2012. This decolouring had been caused mainly by low temperature. Decolouring 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 $^{0}$C had a greater reduction in chlorophyll content rather than 30 $^{0}$C. In the other side, LL leaves had not decolouring symptom during low temperature, it was almost similar with no significance decreasing chlorophyll content of mangrove *Kandelia candel* grown either at 30 or 15 $^{0}$C (Kao *et al*., 2004). Altohugh 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. 2.3). These results 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 decrease the amount of excess excitation energy that has to be dissipated (Burritt and Mackenzie, 2003).

Significant increases in total chlorophyll lead raising in $CO_2$ exchange were due to increased photosynthetic rate (Evans, 1989), as shown in mangrove *A. marina* and *Hibiscus tiliaceus* (Naido *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 HL and ML had higher $P_N$ than LL leaves while PAR increasing (Fig 2.4). In this study, it was found that under light saturating conditions, $g_{max}$ and $E_{max}$ showed similar trends, they are LL<ML<HL respectively (Fig 2.5, Table 2.1). It described that the $P_{max}$ of *R. mucronata* seedlings were 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 (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, that 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, indicate ML effectiveness and also chance to conserve water in better level. It will be useful while ML seedlings adapt with saline condition.

In this study, it was found that the light saturation point of all treatments were commonly at PAR level around 1000 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 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 1000 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 than 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*.

These results that showing different characteristics of $P_N$ responses of *R. mucronata* leaves to light intensity (Fig 2.4) 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 on 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 $^0$C. In contrast, some latter reports indicate that the relationship between the net photosynthetic rate and leaf temperature indicated a wide peak between 29 and 34 $^0$C (Okimoto *et al*., 2007). This finding showed that relationship between $P_{max}$ and leaf temperature indicated a broad peak, which was depending on the pre-condition temperature. At high pre-condition temperatures between August-November 2011 and May-July 2012, $P_{max}$ was obtained between 29-34 $^0$C leaf temperatures, but at lower (23-29 $^0$C) leaf temperatures in the other months (Fig 2.6). Furthermore, the effect of leaf temperature on $P_N$ shows seasonal variation only in those letters which were set at 30 $^o$C correlating with the pre-condition temperature. During the hot months, it was found that LL leaves sustained a better photosynthetic performance while leaf temperature was set at low temperature, 25 $^0$C, as compared to HL and ML leaves (Fig 2.7). 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 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 *in vivo* assessment of biochemical components of leaf photosynthesis, such as ribulose-biphosphate carboxylase (rubisco) activity (Caemmerer and Farquhar, 1981). Furthermore, maximum photosynthetic rate responses to $C_i$ is beneficial to indicate the capacity or potential of leaf photosynthesis. As shown in Fig 2.8, 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, in this study, it was found that seasonal variation of temperature significantly affected P' and $P_{max}$-$C_i$. This result is in agreement with that of Campbell *et al.* (2005) whose findings showed 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 trough 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 were found mainly during cold months (Fig 2.9). Lowering the temperature generally reduces metabolic rates and might 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). The combination of low temperature-high light intensity during cold months might accelerate the damage of 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 2.10A and Table 1) demonstrate the contribution of qP in order to $P_{max}$ achievement level. The response of qP represented the openness of PSII centres (Kitao *et al*., 2003) and high qP was beneficial for the separation of electric charge in reaction centre (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 in $P_N$ resulted a higher qP in plants (Kao and Tsai, 1999).

Moreover, the high qN value of HL leaves on February 2012 (Fig 2.10 B) represented that the using of light energy probably 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 of 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 2.3).

The regular value 0.75 - 0.85 of Fv/Fm ratios have been considered normal for unstressed plants (Hunt, 2003), and decline of Fv/Fm under 0.75 could indicate a disturbance in or damage to the photosynthetic apparatus that due to photoinhibition (Litchtenthaler *et al*., 2005). HL & ML got photoinhibition on February and March 2012 (Fig 2.11), 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 decolouring 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), in this study, but it was found that LL plants sustained low susceptibility for photoinhibition. The Fv/Fm of LL leaves declined during cold months, but the values

were always higher than 0.75 (Fig 2.11) 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 under light (Alves *et al*., 2002). Although the mechanism was not clear, it was 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 photoautotrophs 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 hormon-related genes were significantly affected by the light intensity (Majláth *et al*., 2012).

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, Ci, 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. These findings indicate that seedling grown under moderate shade

condition showed better ability to maintain a high carbon fixation capacity than deep shade condition. 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.

# CHAPTER 3

## An improved method for the simultaneous determination of photosynthetic $O_2$ evolution and $CO_2$ uptake of *Rhizophora mucronata* leaves under aqueous condition

### 1. Introduction

Leaf $O_2$ evolution and $CO_2$ uptake are fundamental mechanisms that support oxygen and carbon ecosystems from the individual plant to the global scale. Based on the photosynthesis chemical formula, which justifies that the ratio of $O_2$ evolution to $CO_2$ fixation is 1:1 (Espie, 1986), the traditional estimation of photosynthetic gas exchange has been evaluated either by $O_2$ evolution or $CO_2$ uptake. However, in an intact leaf, some physiological functions that synthesise and consume $O_2$ and $CO_2$ may vary, particularly under stress conditions (Wu *et al*., 2014), photorespiration (Rosenberg *et al*., 1995) and other oxygenative functions (Taddei *et al*., 2008). This means that the ratio of $O_2$ : $CO_2$ during photosynthesis in intact leaves is not always 1 : 1.

The simultaneous estimation of $O_2$ and $CO_2$ has been done using isotope-Gas Chromatography-Mass Spectrometry (GC-MS) with $^{13}CO_2$ and $^{18}O_2$ (Isobe *et al*., 2011). However, the method is unpopular because the equipment is very expensive (Sipior *et al*., 1996). This study try to improve the potential for a convenient evaluation of $O_2$ evolution and $CO_2$ uptake in photosynthesis by using an $O_2$ electrode and $CO_2$ optodes simultaneously. The main advantages of optodes are that they can be used in non-invasive systems, oxygen and carbon dioxide are not consumed by the optodes, measurements are possible over a wide temperature range, and there is no mechanical stress (Warkentin *et al*., 2007). If this simultaneous

method is convenient, it becomes a useful mechanism to more easily study the physiological effects of photosynthesis.

A simultaneous measurement of $O_2$ evolution and $CO_2$ uptake during photosynthesis is also essential in order to calculate the photosynthetic quotient (PQ), which is described as the molar ratio of the rate of $O_2$ production to the rate of $CO_2$ utilization (Williams and Robertson, 1991). Some ecosystem productivity studies have been made with the assumption that PQ = 1 (Suzumura *et al*., 2002, Nielsen and Nielsen, 2006) that could affect data interpretation of tropical productivity (Taddei *et al*., 2008).

Mangroves represent an important coastal ecosystem in tropical areas. During the seedling stage, the red mangrove (*Rhizophora mucronata* L.) lives periodically in submerged conditions like seaweed or macroalgae. The previous work (Chapter 2; Ulqodry *et al*., 2014) explored the photosynthetic performance of *R. mucronata* leaves using the gas exchange method. This method had a high precision and was rapid (Moore *et al*., 1973; Sobrado, 2005; Okimoto *et al*., 2007), but was limited under aqueous conditions as the Infra-Red Gas Analyser is sensitive to water immersion (Gevaert *et al*., 2011). The advent of a new type of optical electrodes, the so-called opt(r)odes, facilitated the estimation of the *R. mucronata* photosynthetic rate under aqueous conditions. Previous studies have applied optodes for oxygen and carbon independently in the water column, sediments and plant tissues (Gansert *et al*., 2001, Glud *et al*., 2005; Berggren *et al*., 2012).

This study examined the photosynthetic $O_2$ evolution and $CO_2$ uptake rates of *R. mucronata* leaves under aqueous condition. In a simultaneous experiment, a

liquid-phase $O_2$ electrode and $CO_2$ optode was used to demonstrate their interdependence and differences and compared the results with those of the gas exchange method. The determination of PQ values and light-saturated photosynthetic rate ($P_{max}$) of *R. mucronata* under aqueous conditions was investigated.

## 2. Materials and Methods

### Plant materials

Propagules of *R. mucronata* were obtained from a mangrove area on Galang Island, Batam District, Indonesia (0° 45' N, 104° 15' E). Propagules were initially grown in a heated greenhouse at the Laboratory of Tropical Crop Improvement, Saga University, Japan (33° 14' N, 130° 17' E). The fully expanded leaves from 3–4 mangrove seedlings were used as materials.

Leaves were collected early each morning, vacuum-infiltrated with the buffer and stored in the dark until required. One essential consequence of this treatment was the inactivation of rubisco, so that the photosynthetic rates were approximately 10% of those generally observed from leaves taken directly from a plant (Brown, 1998). The leaf sample was sliced into squares of approximately 1 $mm^2$. The leaves were sliced under a 50 mM HEPES buffer containing 0.5 mM $CaSO_4$ and transferred into the electrode chamber that contained the same buffer.

### Simultaneous measurement of photosynthetic rates

Photosynthetic $O_2$ evolution and $CO_2$ uptake were measured simultaneously in a closed chamber using an aqueous phase of a Clark oxygen electrode type polarographic sensor (Hansatech, Norfolk, UK) with a ‘pCO2 mini’ optodes sensor (PreSens GmbH, Regensburg, Germany) that was inserted into the chamber. The

optodes system guarantees a high temporal resolution and a measurement without drift, oxygen consumption, or gas exchange between the incubation chamber and the environment (Warkentin *et al*., 2007). The chamber was equipped with a water jacket to maintain the temperature at 25°C. Periodic checking ensured that the highest illumination intensity did not result in a rapid increase in temperature. Light was provided by a slide projector lamp and the lens system focussed the light into the electrode compartment. The photosynthetically active radiation (PAR) in the chamber was measured with a quantum sensor (model QRT1, Hansatech, Norfolk,UK). It is important that the slices do not obstruct the rotation of the magnetic flea and also the sensor of $pCO_2$ mini. To achieve maximum accuracy, a two point calibration of sensor and buffer was equilibrated with saturation air 21% and also zero oxygen line by using nitrogen bubble. This process also removed any dissolved $CO_2$ from the medium, such that the added $NaHCO_3$ was the only carbon source available.

Photosynthetic $O_2$ evolution and $CO_2$ uptake of *R. mucronata* leaves under aqueous conditions were measured at various pH levels, $NaHCO_3$ concentrations and PAR levels at a temperature of 25°C. The relationship between the pH of the buffer and apparent photosynthetic rate was measured at pH 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0 with $NaHCO_3$ 20 mM as carbon dioxide source under saturated PAR 1,000 μmol $m^{-2}$ $s^{-1}$. The effect of different $NaHCO_3$ concentrations (0, 5, 10, 20 and 40 mM) was measured at pH 7.5 and a saturated PAR of 1,000 μmol $m^{-2}$ $s^{-1}$. In relation to light intensity, PAR values in the chamber were maintained in decreasing levels from 1,000 to 50 μmol $m^{-2}$ $s^{-1}$ by placing various distance between projector lamp and the

chamber. For a dark respiration measurement, the electrode chamber was wrapped in two layers of aluminium foil.

The $O_2$ electrode signal was recorded using Oxygraph Plus System software (Hansatech, Norfolk, UK) as a real-time chart recorder simulation. Simultaneously, the $CO_2$ uptake was measured in the same chamber every 5 s using $pCO_2$ View v1.0.2 software (PreSens GmbH, Regensburg, Germany). There was a lag period less than 2 min for $CO_2$ uptake, and about 3 min for $O_2$ evolution after light activation (Fig 3.1). Generally, lag period of $O_2$ evolution was slightly longer than $CO_2$ uptake but not in significance level. Furthermore, The $O_2$ evolution and $CO_2$ uptake rates were calculated from the initial slopes of the curves during linear photosynthetic activity after lag period finished**.**

As a comparison, the photosynthetic rate based on gas exchange in the air was also performed on leaf pairings similar to those used to measure $O_2$ evolution and $CO_2$ uptake under aqueous conditions. Measurements of leaf gas exchange were conducted using a portable open-flow gas exchange system (LI-6400, Li-COR, Lincoln, NE, USA). The effect of light intensity on the photosynthetic rate was measured from PAR 1000 to 0 μmol $m^{-2}$ $s^{-1}$ (1000, 500, 250, 100, 50, 0 μmol $m^{-2}$ $s^{-1}$) under leaf temperature, VpdL and $CO_2$ input were $25^0$C, 1.7 $\pm$ 0.3 kPa, and 370 μmol $mol^{-1}$, respectively. The light responses of the photosynthetic rate was determined using the rectangular hyperbola model (Okimoto *et al*., 2008) to specify the $P_{max}$ of *R. mucronata* leaves (Ulqodry *et al*., 2014) in air and under aqueous conditions.

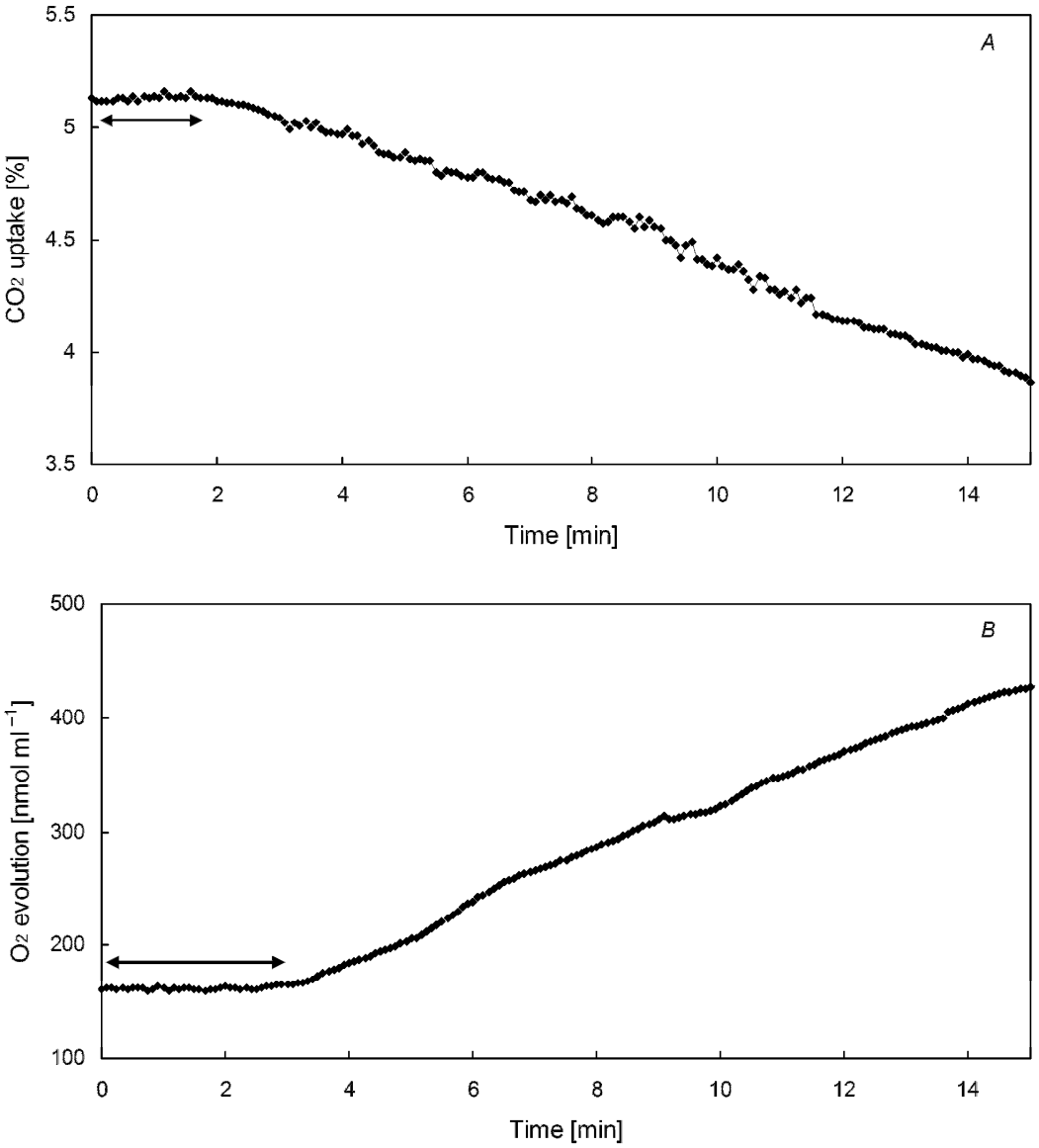


**Fig 3.1** Lag phase (arrow) of photosynthetic $CO_2$ uptake (*A*) and $O_2$ evolution (*B*) of *R. mucronata* leaves as a function of the time after the light was turned on. Data of $CO_2$ uptake were recorded using $pCO_2$ View v1.0.2 software and $O_2$ evolution using Oxygraph Plus System software. Both of concentrations was measured simultaneously in the same chamber every 5 s. The $O_2$ evolution and $CO_2$ uptake rates were calculated from the initial slopes of the curves during linear photosynthetic activity after lag period**.** $CO_2$ (%) and $O_2$ concentration (nmol $ml^{-1}$) will be transformed into net production (µmol $m^{-2}$ $s^{-1}$) using the volume of the enclosure and the related leaf surface that has been introduced to the chamber. The conditions of the medium were temperature $25^0$C, and PAR 1,000 µmol photon $m^{-2}$ $s^{-1}$.

**Statistical analysis**

Analysis of variance (ANOVA) was performed using statistiXL Version 1.x. Significant differences between treatments were further evaluated using the Tukey HSD test ($P<0.05$).

**3. Results**

This study began the experiment by comparing the most suitable leaf shape resulted the highest $O_2$ evolution and $CO_2$ uptake, between small slice pieces (1 $mm^2$) and a larger, chip shape (1 $cm^2$). The results showed that a small *R. mucronata* leaf sample had significantly had higher $O_2$ evolution and $CO_2$ uptake rates compared with the larger, chip shape (Fig. 3.2).

The most important factors for measuring net photosynthetic rate ($P_N$) in aqueous conditions are the pH and carbonate system of the reaction mixture. The $P_N$ in response to pH exhibited a similar pattern for both $O_2$ evolution and $CO_2$ uptake, with higher rates associated with intermediate pH values of 7.0–7.5 compared to low and high pH (Fig. 3.3).

Variation in $P_N$ responses to $NaHCO_3$ concentrations also showed almost similar trends for both $O_2$ evolution and $CO_2$ uptake. The $P_N$ increased with higher $NaHCO_3$ concentrations until reaching the saturation point at 20 mM (Fig. 3.4).

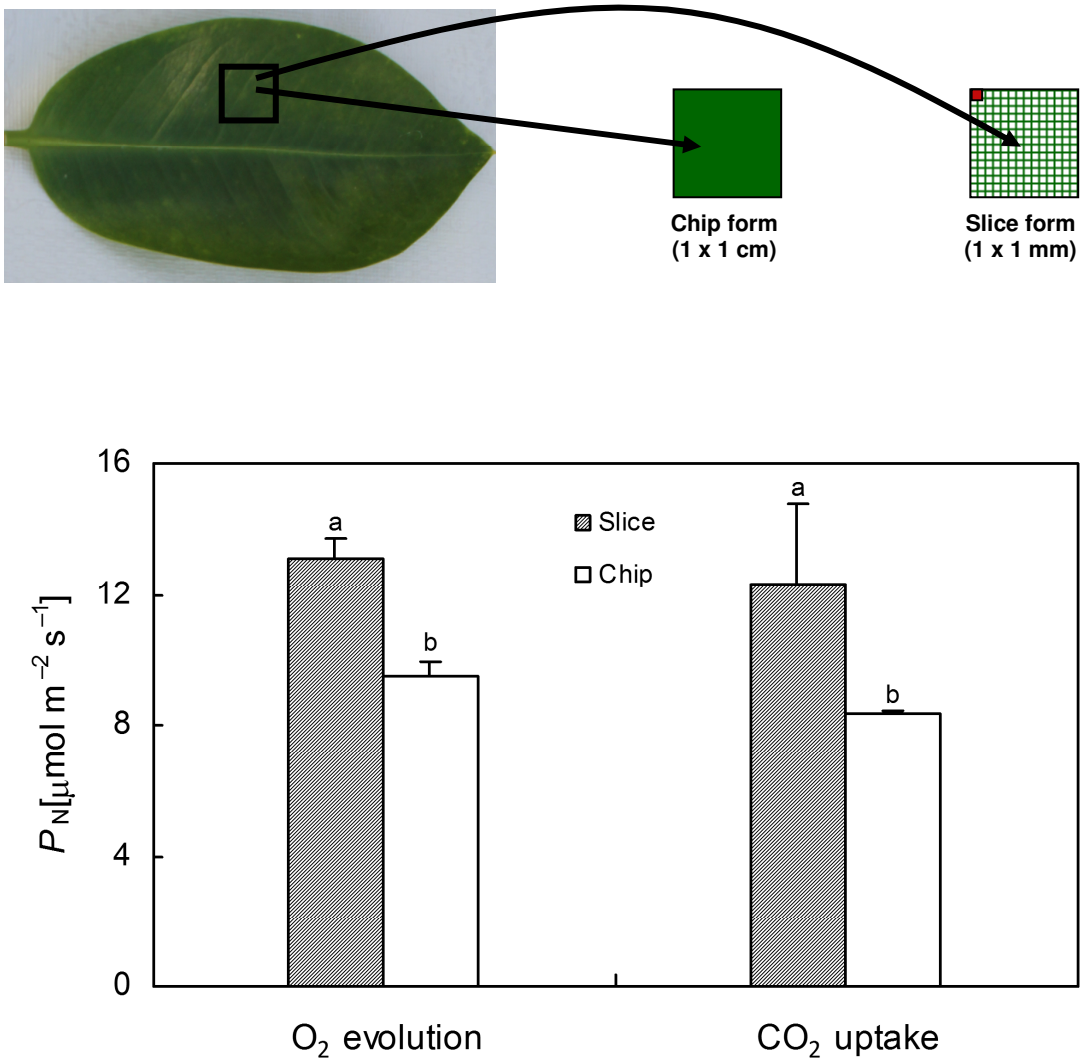


**Fig. 3.2** Photosynthetic $O_2$ evolution and $CO_2$ uptake of *R. mucronata* leaves while measured in slice (1 mm$^2$) and chip (1 cm$^2$) form. The conditions of the medium were temperature 25 $^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM, and PAR 1000 µmol photon m$^{-2}$ s$^{-1}$. Value is mean $\pm$ SD (n=3-4 plants). Different letters over bars represent significant differences among all treatments ($P<0.05$; Tukey HSD's test)

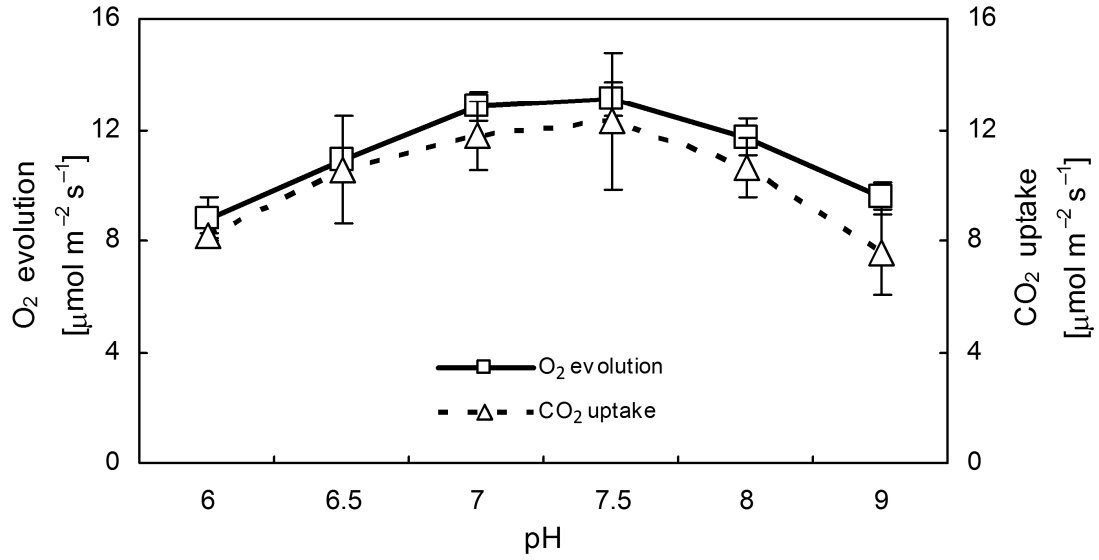


**Fig. 3.3** Photosynthetic $O_2$ evolution and $CO_2$ uptake of *R. mucronata* leaves at various pH levels. The conditions of the medium were temperature 25 $^0$C, $NaHCO_3$ 20 mM, and PAR 1000 μmol photon $m^{-2}$ $s^{-1}$. Value is mean $\pm$ SD (n=3-4 plants).

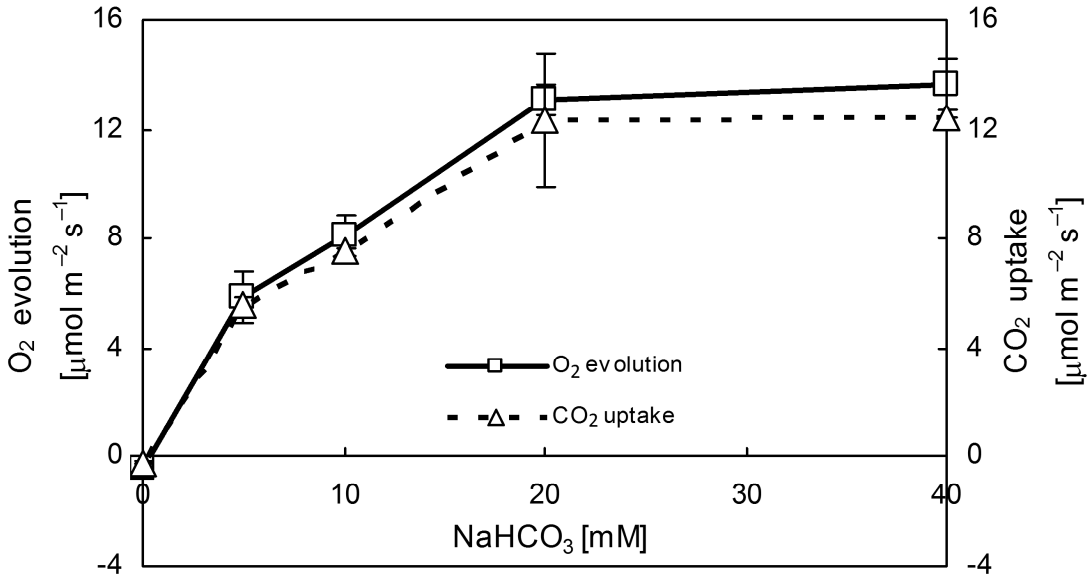


**Fig. 3.4.** Photosynthetic $O_2$ evolution and $CO_2$ uptake of *R. mucronata* leaves at various $NaHCO_3$ concentrations. The leaf sample was cut into slice form. The conditions of the medium were temperature 25 $^0$C, Buffer pH 7.5, and PAR 1000 µmol photon $m^{-2}$ $s^{-1}$. Value is mean $\pm$ SD (n=3-4 plants)

The interesting finding was that although there was no significant difference between $O_2$ evolution and $CO_2$ uptake, $O_2$ evolution values were always higher than $CO_2$ uptake values under the different pH and $NaHCO_3$ concentrations. This result is important for exploring the PQ of *R. mucronata* leaves under aqueous conditions. To be useful, PQ should be determined using the net rate of $O_2$ involved per $CO_2$ fixed simultaneously and can be described as;

$$PQ = \frac{O_2\ evolution}{CO_2\ uptake}$$

The PQ values of *R. mucronata* leaves in different pH and $NaHCO_3$ concentrations ranged from 1.04–1.28 with no significant difference among them (Fig. 3.5).

In order to characterise the functioning of photosynthetic apparatus of *R. mucronata* in air and aqueous conditions, the light curves of $P_N$ for similarly paired leaves were estimated. In Fig. 3.6, at low light levels (PAR < 500 μmol photons $m^{-2}$ $s^{-1}$), the photosynthetic rate of $O_2$ evolution and $CO_2$ uptake under aqueous conditions was lower than the photosynthetic $CO_2$ exchange in air. However, under light saturation condition, all experiments produced comparable results with similar $P_{max}$ values of 13.37, 13.11 and 12.31 μmol $m^{-2}$ $s^{-1}$ for $CO_2$ exchange in air, $O_2$ evolution under aqueous conditions and $CO_2$ uptake under aqueous conditions, respectively.

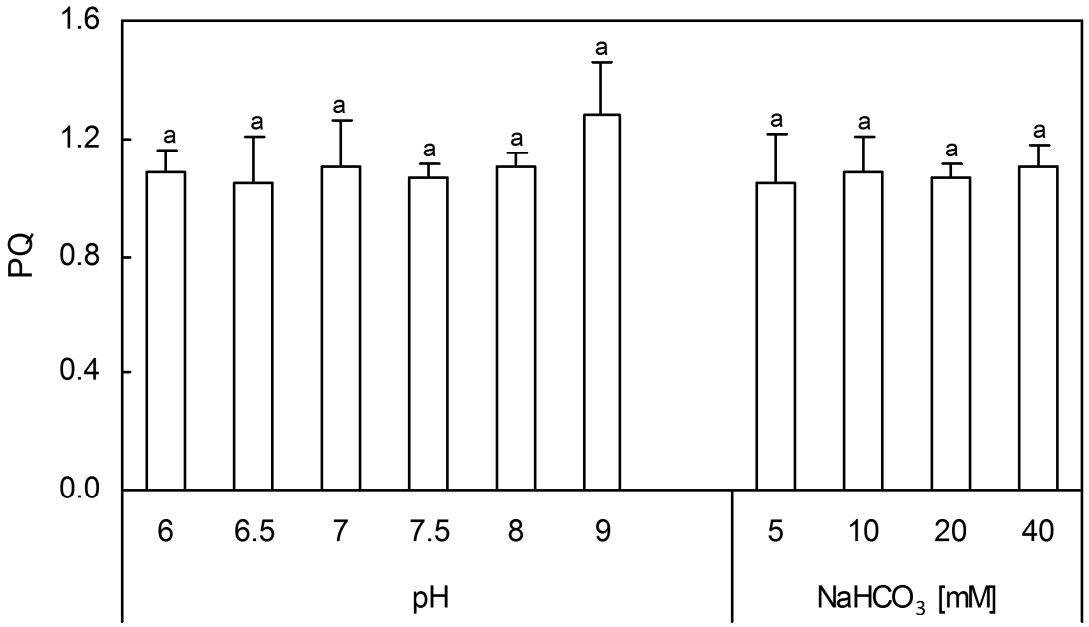


**Fig. 3.5** Photosynthetic quotients (PQ) in different pH and $NaHCO_3$ concentration under aqueous conditions. They were measure at saturating level of PAR 1000 µmol photons $m^{-2}$ $s^{-1}$ and chamber temperature 25 $^0$C. Values noted with the same letter for a particular treatments are not significantly different at $\alpha$ = 0.05 (Tukey HSD's test).

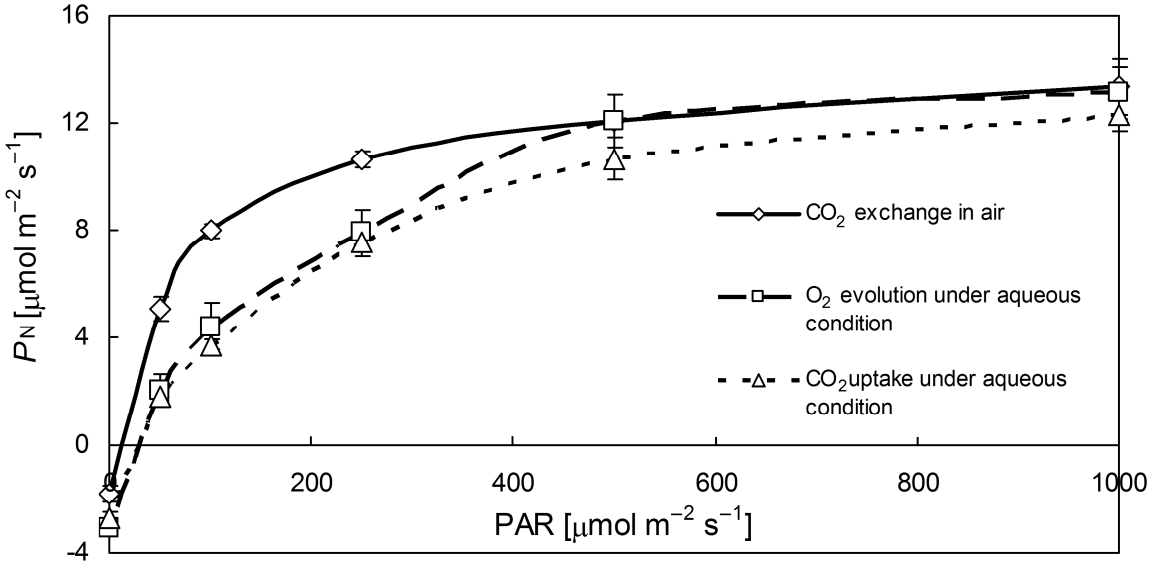


**Fig. 3.6** Response of net photosynthetic rate ($P_N$) to increasing PAR in the *R. mucronata* leaves. Measurements in air were made with a portable open-flow gas exchange system, LI-6400 ($CO_2$ exchange in air) and measurements under aqueous condition were made simultaneously with an aqueous-phase $O_2$ electrode ($O_2$ evolution) and '$pCO_2$ mini' optode system ($CO_2$ uptake). Temperature was $25^0$C for all measurements. Value is mean $\pm$ SD.

## 4. Discussion

Previous results indicated that cutting leaves into small pieces can be negligible during $O_2$ evolution measurement under aqueous condition (Kawamitsu and Boyer, 1999). In this study, it was shown that a small *R. mucronata* leaf sample had significantly had higher $O_2$ evolution and $CO_2$ uptake rates compared with the larger, chip shape. It suggests that slicing the leaf tissues facilitates the increasing of gas exchange across the boundary layer at the tissue surface (Brown, 1998). This eliminates the effect of stomatal resistance for $CO_2$ diffusion, and free $CO_2$ molecules or $HCO_3^-$ ions may penetrate more easily into the tissue of the leaf slice, resulting in a higher photosynthetic rate (Ishii *et al*., 1977).

Dissolved $CO_2$ in water occurs in three inorganic forms, free aqueous carbon dioxide (free $CO_2$), bicarbonate ($HCO_3^-$) and carbonate ions ($CO_3^{2-}$). If the equilibrium is affected by a change in pH, this could potentially influence $P_N$ (Riebesell *et al*., 2007). Under a high pH condition of 8.0–9.0, free molecular $CO_2$ decreased and bicarbonate increased (Chen and Durbin, 1994). This meant that the free $CO_2$ in the reaction mixture became limiting, reducing $P_N$. The $P_N$ is higher under intermediate pH values of 7.0–7.5 compared with low and high pH also demonstrated that the main carbon utilised as the substrate for *R. mucronata* leaf photosynthesis was free $CO_2$ molecules rather than bicarbonate. Almost all terrestrial plants use only free $CO_2$ for photosynthesis, however, many seaweeds or macroalgae use both free $CO_2$ and external bicarbonate in water as a source of carbon for photosynthesis (Kawamitsu and Boyer 1999; Pierini and Thomaz, 2004). However, the photosynthetic rate to be detected even at pH 9, indicated that *R. mucronata* leaves used bicarbonate as an additional source of carbon under low free $CO_2$

conditions. The requirement of Photosystem II (PSII) for bicarbonate (carbonate) has been observed for intact leaves, isolated thylakoids and PSII-enriched membrane fragments from oxygenic photosynthesisers (Shevela *et al*., 2012). Bicarbonate is required for the regulation of photosynthetic electron transport on the acceptor side of PSII (Wydrzynski and Govindjee, 1975), and is probably also involved in the mechanism of $O_2$ evolution on the oxidising side of PSII (Stemler, 2002).

This study was unable to demonstrate that high free $CO_2$ under low pH condition (< 7.0) resulted in a high $P_N$. It described that leaf $O_2$ evolution and $CO_2$ uptake were strongly related to leaf intracellular conditions. Berge *et al*. (2010) pointed out that as the pH dropped the $H^+$ concentration increased which may affect intracellular pH, which may affect intracellular pH, membrane potential, energy partitioning, and enzyme activity. For this reason, aqueous acidification may reduce $P_N$ through direct pH effects.

The $P_N$ increased with higher $NaHCO_3$ concentrations until reaching the saturation point at 20 mM. The high bicarbonat saturation point indicated that this method need very high carbondioxide source. Particularly in submerged plants, $P_N$ may be limited by a low availability of dissolved inorganic carbon (Maberly and Spence, 1983; Adamec, 1997).

The important result of this study is the ability to explore the PQ values of *R. mucronata* leaves under aqueous conditions. The PQ values of *R. mucronata* leaves in different pH and $NaHCO_3$ concentrations ranged from 1.04 to 1.28 with no significant difference among them. Stoichiometrically, a PQ value equal to unity which means PQ = 1.00 (Rosenberg *et al*. 1995). If this simple photosynthesis

physiology was replaced by an ecological summation of protoplasm production, including carbohydrates, protein, lipids, and nucleic acids, then the theoretical PQ would be higher (Williams and Robertson, 1991). Theoretical PQ values typically range from 1.0–1.3 (Rosenberg *et al*., 1995), or up to 1.4 (Laws, 1991).

Purely based on stoichiometric and theoretical considerations of PQ values, results similar or higher than unity would be expected. A PQ of 1.0 infers that the sole product of photosynthesis is carbohydrate and a PQ > 1.0 indicates that more reduced compounds are produced, such as fats and proteins (Chisholm, 1998). This result also suggested that the simultaneous measurement of $O_2$ evolution and $CO_2$ uptake by using a Clark oxygen electrode type polarographic sensor and 'pCO2 mini' optodes sensor provided a simple, stable and precise measurement of net PQ under aqueous conditions.

The net PQ values in all measurements was never less than unity. PQ values of more than unity were expected in macrophyta (Rosenberg *et al*., 1995). PQ values also might useful to show the photorespiration occurred or not in intact leaves under aqueous conditions. A possible explanation for a PQ to be not unity would be photorespiration (glycolate production) as a result of oxygenase activity of ribulose-1,5-bisphosphate (RuBP) carboxylase at high ambient oxygen concentrations (Rosenberg *et al*., 1995). Photorespiration occurs when rubisco, which principally functions as carboxylase, is substituted by the oxygenase function (Taddei *et al*., 2008). In terrestrial $C_3$ plants, photorespiratory consumption of $O_2$ can account for 25% of rubisco activity (Falkowski and Raven, 1997). Conversely, photorespiration is assumed to be of minor importance to aquatic plants compared with terrestrial $C_3$ plants (Laws *et al*., 2000), because submerged environmental conditions, such as

fairly constant oxygen and total inorganic carbon concentrations, does not favour photorespiration (Rosenberg *et al*., 1995).

At low light levels, the photosynthetic $O_2$ evolution and $CO_2$ uptake under aqueous conditions was lower than the photosynthetic $CO_2$ exchange in air. This result is likely to be related to the reduction of low light utilisation while the leaf slices were rotated under aqueous conditions. Another possible explanation was this study worked well under light saturation compared with light limitation. Therefore, In future, it's need to improve the simultaneous measurements of photosynthetic $O_2$ evolution and $CO_2$ uptake under aqueous conditions in low light conditions.

Furthermore, the light saturation points for all $P_N$ measurements ($CO_2$ exchange in air, $O_2$ evolution under aqueous condition and $CO_2$ uptake under aqueous condition) were similar at PAR levels around 500–1,000 μmol photons $m^{-2}$ $s^{-1}$. The $P_{max}$ which demonstrates the potential photosynthetic capacity of *R. mucronata* leaves (Chapter 2; Ulqodry *et al*., 2014), was also determined. All experiments produced comparable results with similar $P_{max}$ values of 13.37, 13.11 and 12.31 μmol $m^{-2}$ $s^{-1}$ for $CO_2$ exchange in air, $O_2$ evolution under aqueous conditions and $CO_2$ uptake under aqueous conditions, respectively. In comparation with gas exchange, the maximum photosynthetic rate in photosynthetic $O_2$ evolution and $CO_2$ uptake under aqueous condition was achieved under very high carbon dioxide condition. The $P_{max}$ value and daily period of irradiance when plants were in the water and air would be useful as an indicator of primary production (Zimmerman *et al*., 1994). The similar $P_{max}$ values suggested that all treatments resulted in a high capacity to adjust the photosynthetic apparatus under light saturation conditions.

# CHAPTER 4

## Photosynthetic $O_2$ evolution and $CO_2$ uptake responses on short-term impacts of NaCl concentrations and soaking periods to mangrove leaves

### 1. Introduction

Mangrove is a major and unique coastal ecosystem in tropic area. They have a higher carbon fixation capacity than terrestrial forests (Lugo and Snedaker, 1974; Donato *et al*., 2011; Okimoto *et al*., 2013), adaptation ability under abiotic stress (McLeod and Salm, 2006), and specific habitat zonation (Bunt, 1996; Youssef and Saenger, 1999). Mangroves, which thrive luxuriantly in tidal saline wetlands, are especially adapted to salinity and submerged stresses (Naidoo *et al*., 1997).

Striker (2012) classified submerged condition in waterlogging, partial flooding and complete flooding condition. Under waterlogging condition, only the root system of plant is under the anaerobic conditions, while the shoot and leaves are under atmospheric normal conditions. Under partial flooding conditions, plants have a portion of their shoots underwater, besides having their roots completely immersed in water-saturated soil. Under complete flooding, plants confront the most stressful condition because all plant compartments (including leaves) are underwater. During complete flooding condition, the chances of plant to capture atmospheric oxygen and to continue with carbon fixation are restricted. This situation is worsened due to the irradiance available to sustain underwater photosynthesis for survival is drastically reduced (Colmer *et al*., 2011; Vashist *et al*., 2011). Furthermore, in this study the term of "soaking condition" was used to reflect the complete flooding condition whereas the leaves usually immersed in water column.

Belong to the $C_3$ plant, mangroves also can be classified as "seaweed", since it can grow in soaking and high salinity conditions, whereas $C_3$ plants could not survive (Kawamitsu *et al*., 2003[a]). In recent decades, many workers have been interested in understanding how stress limits mangrove photosynthesis (Naidoo *et al*., 1997; Krauss *et al*., 2006; Ulqodry *et al*., 2014). However, there are relatively few studies on the combined effects of salinity and soaking conditions in mangrove photosynthetic performance (Cardona-Olarte *et al*., 2006).

Species differences in mangrove responses to the interactive effects of some stress conditions might explain important differences in mangrove forest structure (Krauss *et al*., 2008). Mangroves might exhibit distinctions in photosynthetic capacity and sensitivity to environmental conditions for different species (Ball, 1986). *Bruguiera gymnorrhiza*, *Rhizophora mucronata* and *Avicennia marina* are three major mangrove species in Indonesia, and are dominant along east Sumatera coastlines. Based upon inferences made from intertidal distributions, these three species appear to differ in their sensitivity to salinity and flooding on chloroplast, tissue water potential and ion concentration (Naidoo, 1985). Nevertheless, the differences in responses to salinity and soaking conditions on photosynthetic performance have not been well known among these three species. The mangrove photosynthetic responses as combined soaking-salinity effects may useful to clarify the mangrove zonation pattern.

Accurately quantifying the performance of aquatic photosynthetic plant is fundamental in developing our understanding of energetics and tropho-dynamics in coastal ecosystems (Gevaert *et al*., 2011). The estimation of mangrove

photosynthetic gas exchange has been evaluated either by $O_2$ evolution or $CO_2$ uptake (Clough and Sim, 1989; Sobrado, 2005; Okimoto *et al*., 2007) but was limited under flooding conditions as the Infra-Red Gas Analyser is sensitive to water immersion (Gevaert *et al*., 2011). This study has examined the simultaneous measurements of mangrove leaf $O_2$ evolution and $CO_2$ uptake under aqueous condition using the leaf-disc $O_2$ electrode with $CO_2$ optodes sensor. This method provided a simple and stable measurement of net photosynthetic quotient (PQ) under aqueous conditions (Chapter 3; Ulqodry *et al*., 2015).

The PQ values represent the molar ratio of the rate of $O_2$ production to the rate of $CO_2$ uptake (Williams and Robertson 1991). This value provides fundamental information on metabolic pathways (Taddei *et al*., 2008), balanced growth (Davies *et al*., 2003) and useful to clarify the primary productivity of an ecosystem (Lee and Bong 2006). In this study, it was hypothesised that PQ value may explain important distinctions among these three mangrove species in order to clarify their adaptation ability to soaking and salinity conditions.

The purpose of this study was to determine the photosynthetic $O_2$ evolution and $CO_2$ uptake performances of three mangrove species, *i.e*., *B. gymnorrhiza*, *R. mucronata* and *A. marina* to salinity and soaking periods. Their separately and interaction photosynthetic responses were then compared to the characteristic habitat of each species in the mangrove zonation.

## 2. Materials and Methods

### Plant materials

Propagules of *B. gymnorrhiza* and *R. mucronata* were collected from a mangrove area of Galang Island in Batam District, Indonesia (0° 45' N, 104° 15' E) and *A. marina* from Banyuasin peninsula, Indonesia ($02^0$ 11' S, $104^0$ 53' E). All propagules were raised in the greenhouse with heating system at the Laboratory of Tropical Crop Improvement, Saga University, Japan (33° 14' N, 130° 17' E). Plants were watered to ensure that drought did not confound experimental results.

Plants were subjected to treatments after 5 months. The fully developed healthy leaves from upper side of 3-4 mangrove seedlings were used as main materials. In order to resemble the complete flooding condition, at first, the intact leaves were immersed to water column and assigned randomly to one of 12 treatment combinations of NaCl concentrations and soaking periods:

| NaCl Concentration (salinity level) | Soaking Periods (min) | | | |
|---|---|---|---|---|
| | 15 | 30 | 60 | 120 |
| 100 mM (low) | √ | √ | √ | √ |
| 300 mM (mid) | √ | √ | √ | √ |
| 500 mM (high) | √ | √ | √ | √ |

Especially for control leaves, they were not soaked and no NaCl added. 500 mM NaCl equivalent to seawater salinity level (Robinson, 1988; Hariadi *et al*., 2011; Eisa *et al*., 2012). After the soaking and NaCl treatments, the leaf sample is sliced using a safety razor under 50 mM HEPES buffer containing 0.5 mM $CaSO_4$ (Ishii *et al*., 1977), vacuum-infiltrated with the buffer and transferred into the electrode chamber containing the same buffer.

**Photosynthetic rates**

Photosynthetic rates of mangrove leaves was representated by photosynthetic oxygen evolution and carbon dioxide uptake. Both of them was measured simultaneously in a closed chamber using an liquid phase of a Clark oxygen electrode polarographic sensor (Hansatech, Norfolk, UK) with a sensor of 'pCO2 mini' optodes system (PreSens GmbH, Regensburg, Germany) and the details were described in Ulqodry *et al*. (2015, Chapter 3). Light was provided by a slide projector lamp and calibrated with a quantum sensor (model QRT1, Hansatech, UK) in order to determine the amount of photosynthetically active radiation (PAR). All measurements were carried out at 25°C with 20 mM $NaHCO_3$ as carbon dioxide source. The rates of $O_2$ evolution and $CO_2$ uptake were calculated from the initial slopes of the curves during the periods of apparent linear photosynthetic activity**.**

In order to determine the photosynthetic performance of mangrove leaves at light response, various PAR levels was maintained in decreasing order from 1000 to 50 μmol $m^{-2}$ $s^{-1}$ by placing various distance between projector lamp and the chamber. The rectangular hyperbola model was used to find the maximum photosynthetic rate ($P_{max}$) and initial slope as the response of photosynthetic rate to light intensity for each mangrove species (Okimoto *et al*., 2008; Ulqodry *et al*., 2014; Chapter 2). For a dark respiration measurement, the electrode chamber was wrapped in two layers of aluminium foil.

**Photosynthetic Quotient (PQ)**

PQ could be determined using the net rate of $O_2$ involved per $CO_2$ fixed simultaneously and can be described as (Ulqodry *et al*., 2015, Chapter 3),

$$PQ = \frac{O_2\ evolution}{CO_2\ uptake}$$

**Statistical analysis**

Statistical analysis was performed by Analysis of variance (ANOVA) using statistiXL Version 1.x. When differences between treatments were significant according to the ANOVA analysis, the difference between means was evaluated using Tukey HSD's test ($P < 0.05$).

## 3. Results

**Photosynthetic-light response of mangrove leaves subjected to variation of NaCl concentrations and soaking periods**

The light saturation points of all treatments were commonly at PAR level around 500-1000 μmol photon $m^{-2}$ $s^{-1}$ (Figs. 4.1 – 4.3). Under control condition (no soaking and no NaCl added), the maximum photosynthetic oxygen evolution of *R. mucronata* had the higher rate (13.10 μmol $m^{-2}$ $s^{-1}$) than *B. gymnorrhiza* (11.92 μmol $m^{-2}$ $s^{-1}$) and *A. marina* (11.05 μmol $m^{-2}$ $s^{-1}$). However, while subjected to various NaCl concentrations and soaking periods, the maximum photosynthetic rate responses to light intensity on three mangrove species showed different responses (Fig 4.4).

Soaking periods had great effect on reducing of photosynthetic $O_2$ evolution and $CO_2$ uptake in *B. gymnorrhiza* (Figs. 4.1 and 4.4). During soaking periods, maximum photosynthetic rate of *B. gymnorrhiza* also decreased simultaneously with salinity escalation, in which case the decreasing of maximum photosynthetic $O_2$ evolution was higher than maximum photosynthetic $CO_2$ uptake. Under high salinity (NaCl 500 mM), photosynthetic $O_2$ evolution and $CO_2$ uptake of *B. gymnorrhiza* reached the lowest rate compared with other species while exposed to high PAR 1000 μmol photon $m^{-2}$ $s^{-1}$.

For *R. mucronata*, Figs 4.2 and 4.4 showed that soaking periods under low (NaCl 100 mM) and mid salinity (NaCl 300 mM) had no significance effect on photosynthetic performance and maximum photosynthetic $O_2$ evolution and $CO_2$ uptake. This result indicated that *R. mucronata* adapted up to moderate salinity and soaking condition better than *B. gymnorrhiza*. However, photosynthesis of *R. mucronata* leaves also declined as under high salinity, namely the maximum photosynthetic $O_2$ evolution decreased more clearly than $CO_2$ uptake.

In contrast with *B. gymnorrhiza* and *R. mucronata*, photosynthetic performance and maximum photosynthetic capacity of *A. marina* in high salinity was higher than control during soaking periods (Figs. 4-3 and 4-4). Under soaking conditions with low and mid salinity, photosynthetic-light responses of *A. marina* had similar pattern with *R. mucronata* and also adapted better than *B. gymnorrhiza*.

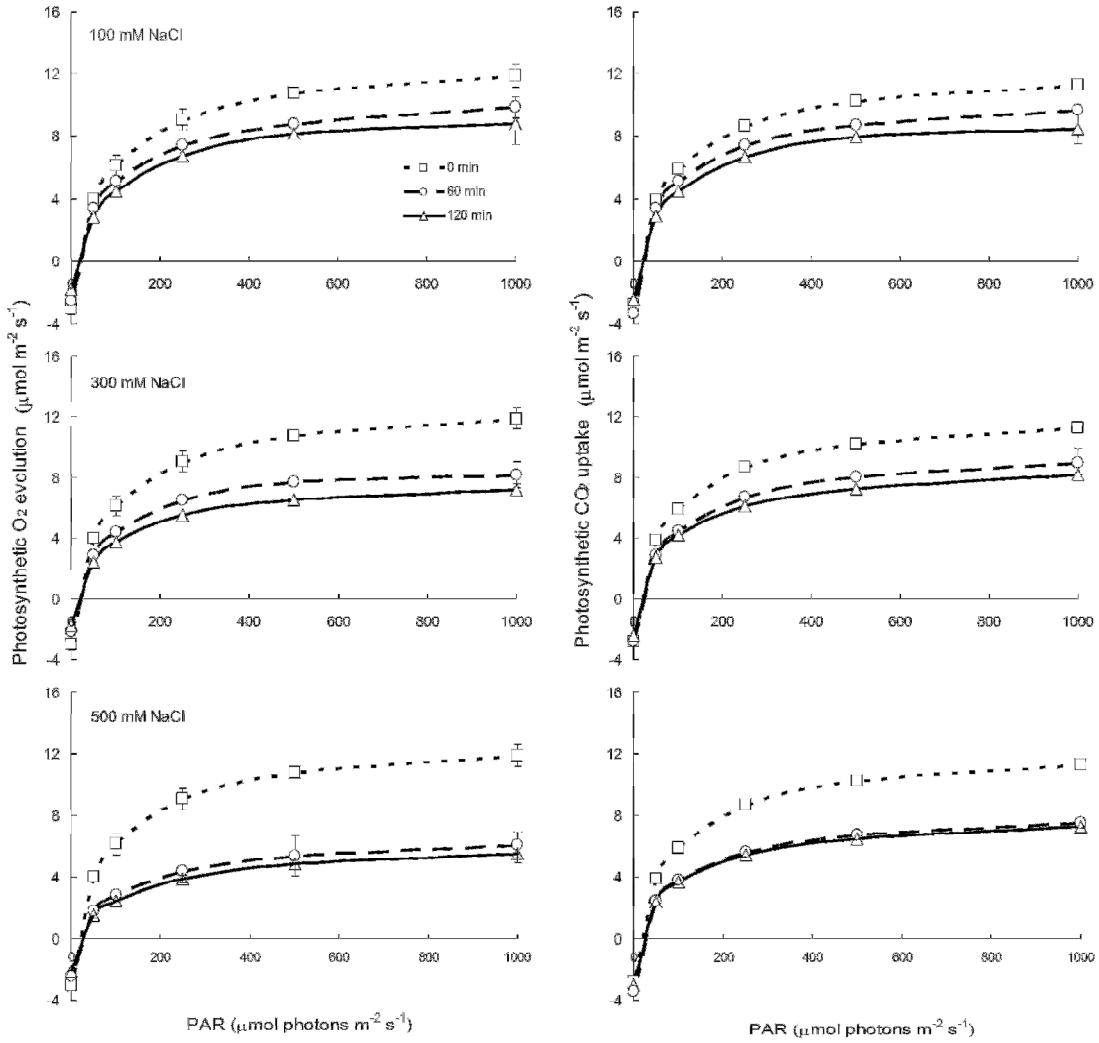


**Fig. 4.1** Light response curves for photosynthetic $O_2$ evolution and $CO_2$ uptake of mangrove leaves *B. gymnorrhiza* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Conditions of the medium was temperature $25^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. Value is mean $\pm$ SD (n=3-4 plants).

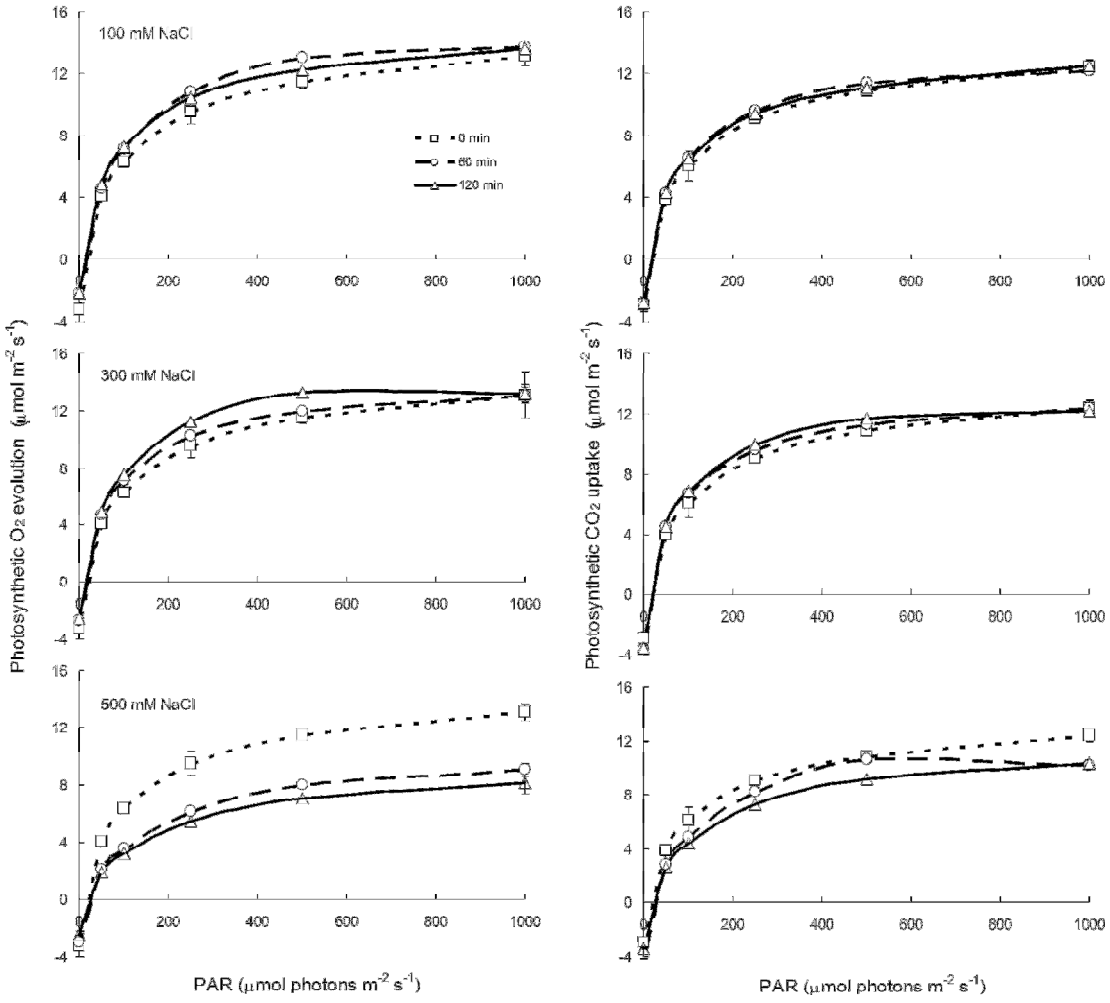


**Fig. 4.2** Light response curves for photosynthetic $O_2$ evolution and $CO_2$ uptake of mangrove leaves *R. mucronata* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Conditions of the medium was temperature $25^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. Value is mean $\pm$ SD (n=3-4 plants).

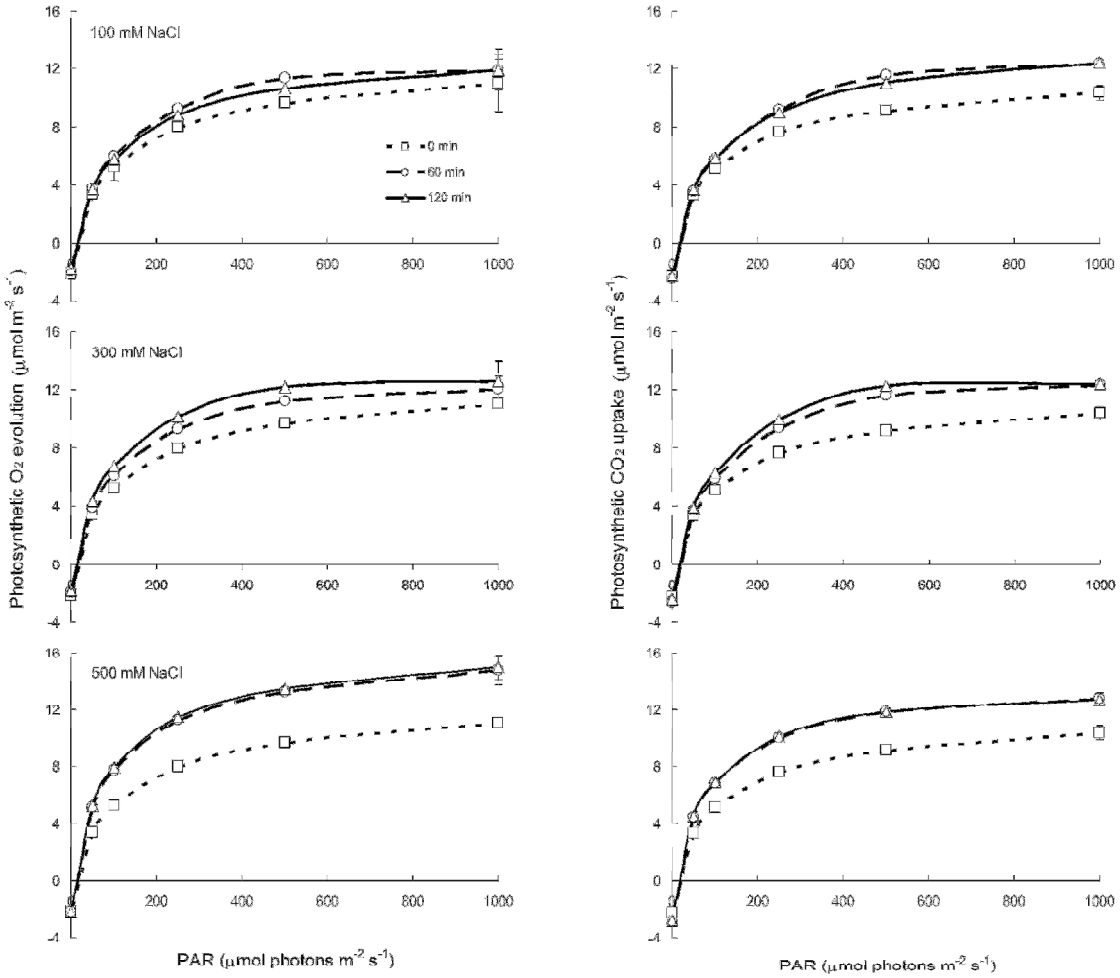


**Fig. 4.3** Light response curves for photosynthetic $O_2$ evolution and $CO_2$ uptake of mangrove leaves *A. marina* subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Conditions of the medium was temperature $25^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. Value is mean $\pm$ SD (n=3-4 plants).

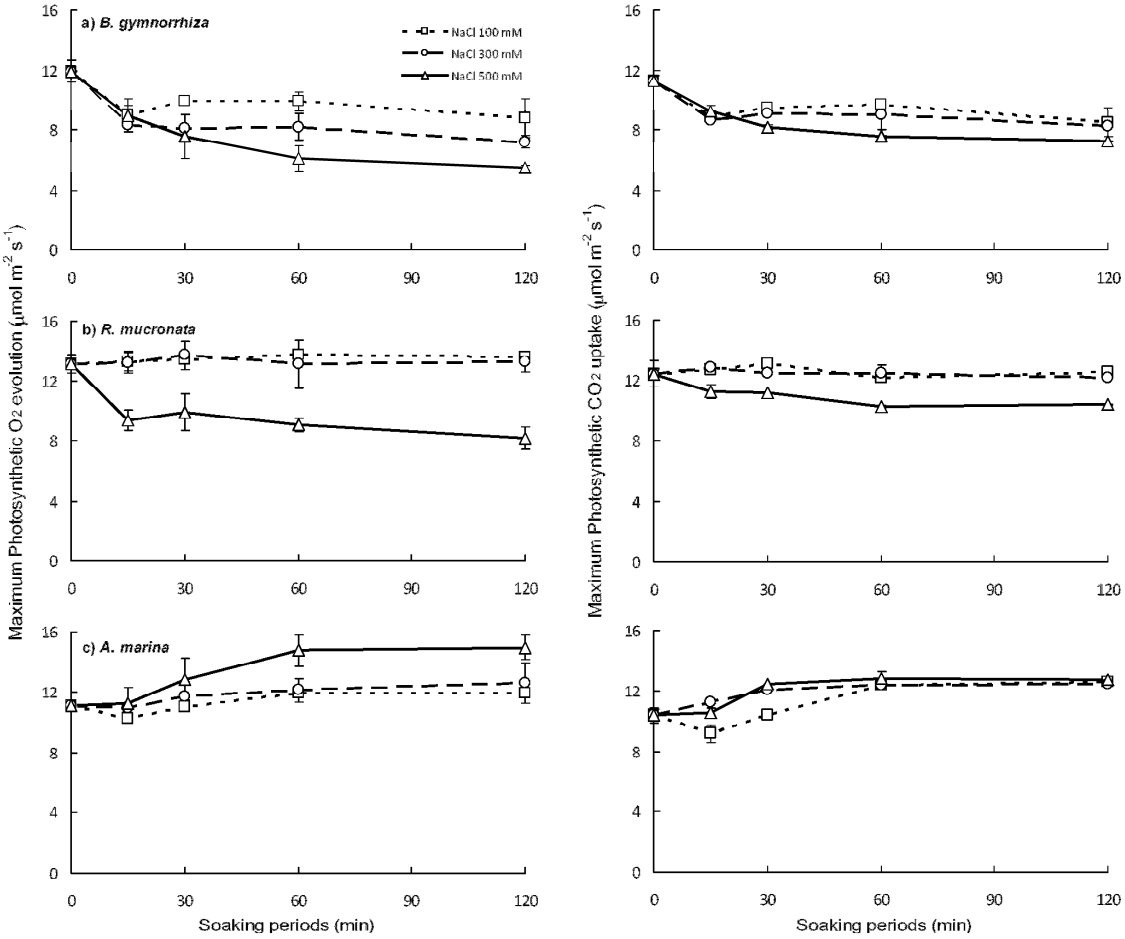


**Fig. 4.4** Effects of soaking periods and NaCl concentrations on maximum photosynthetic $O_2$ evolution and $CO_2$ uptake in mangrove species. Conditions of the medium was temperature 25 $^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. Value is mean $\pm$ SD (n=3-4 plants).

Furthermore, the initial slope of photosynthetic-light response as the estimation of quantum yield (Leverenz *et al*., 1990) or quantum efficiency at low irradiance (Akhkha, 2010) is displayed in Fig 4-5. Initial slope of photosynthetic-light responses had almost similar pattern with maximum photosynthetic rate for all treatments of each mangrove species. The initial slope of light response of *B. gymnorrhiza* leaves decreased with increasing soaking period and NaCl concentration. *R. mucronata* had an high initial slope to low and mid NaCl concentration during soaking periods. Among those three mangrove species, initial slope of photosynthetic $O_2$ evolution and photosynthetic $CO_2$ uptake in *A. marina* was uniquely increased as compared with other species.

Generally, photosynthetic $CO_2$ uptake had similar tendency with photosynthetic $O_2$ evolution, but in different values. This result is important to elucidate the PQ values of three mangrove leaves subjected to salinity and soaking conditions.

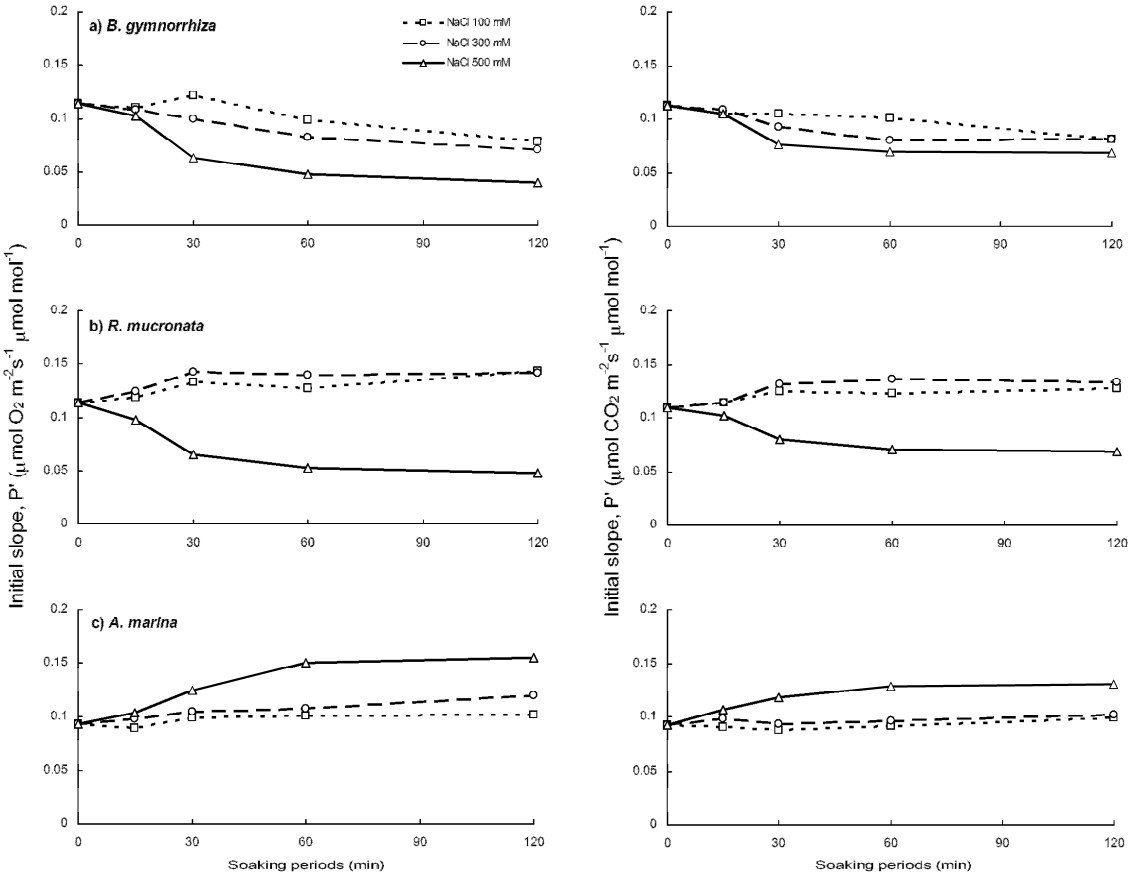


**Fig. 4.5** Effects of soaking periods and NaCl concentrations on initial slope photosynthetic-light responses (quantum yield) of $O_2$ evolution and $CO_2$ uptake in mangrove species. Conditions of the medium was temperature 25 $^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. Value is mean $\pm$ SD (n=3-4 plants).

**Photosynthetic Quotient (PQ)**

The PQ values as effects of soaking period and NaCl concentration for *B. gymnorrhiza, R. mucronata* and *A. marina* were range 0.76–1.04, 0.78–1.13, and 0.96–1.18 respectively (Figs 4.6 – 4.8). PQ < 1 was found in *B. gymorrhiza* leaves soaked under both mid and high salinity condition and difference significantly with control (Fig 4.6). Although under low salinity during soaking periods the photosynthetic $O_2$ evolution and photosynthetic $CO_2$ uptake of *B. gymnorrhiza* declined, but its PQ values was almost not change around 1 (Figs 4.1, 4.4 and 4.6).

In *R. mucronata*, PQ < 1 was found mainly in leaves soaked under high salinity, while soaking periods with low and mid salinity were not (Fig 4.7). This results also indicated that *R. mucronata* might adapted well to low and mid NaCl concentration during soaking periods.

Figure 4.8 showed that PQ < 1 was almost not found in *A. marina* leaves under all saline soaking conditions. PQ <1 occurred while *A. marina* leaves exposed in low salinity but not in significance level. *A. marina* indicated the opposite tendency compared with *B. gymnorrhiza* and *R. mucronata* in respons to soaking and salinity conditions.

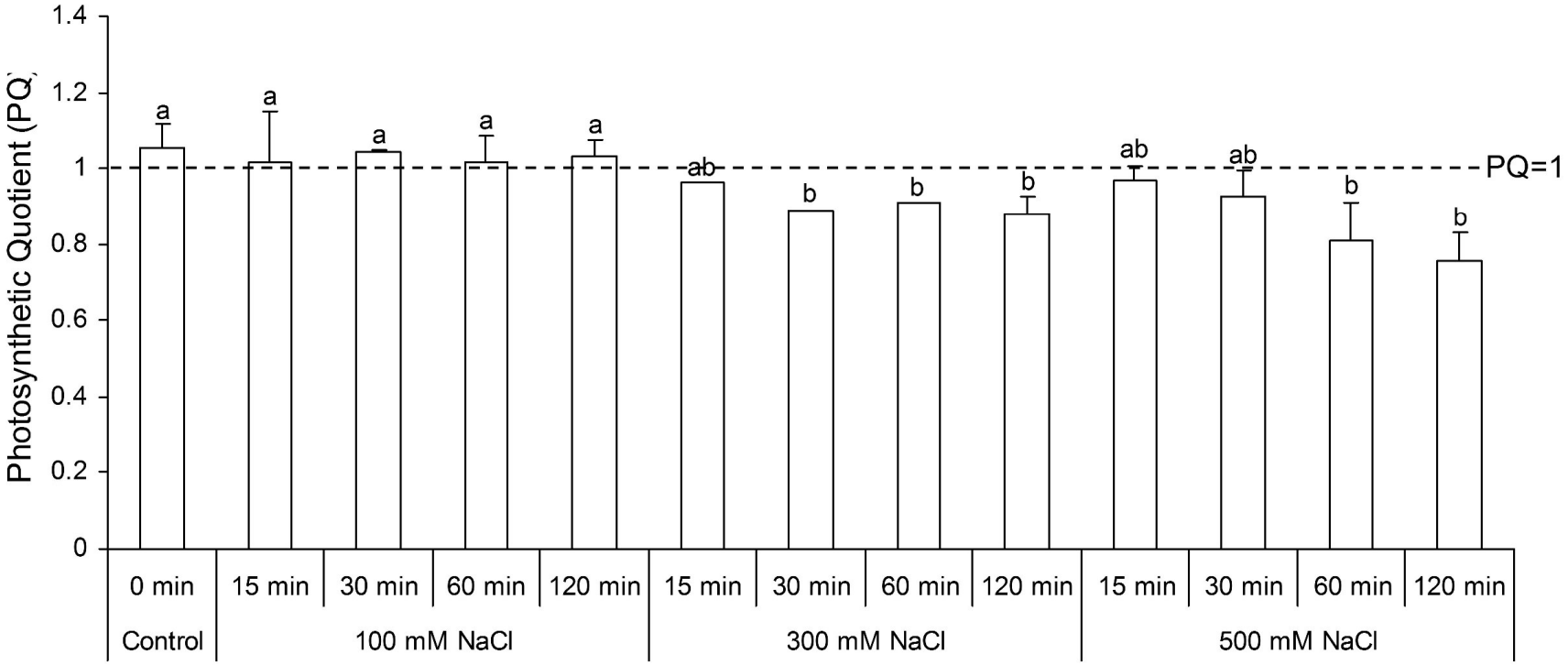


**Fig. 4.6** Photosynthetic quotients (PQ) of mangrove *B. gymnorrhiza* leaves subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added.Conditions of the medium was temperature $25^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. The standard error is shown above each bar. Values that are significantly different in the control and same NaCl concentrations ($P < 0.05$; Tukey HSD test) are indicated by different letters.

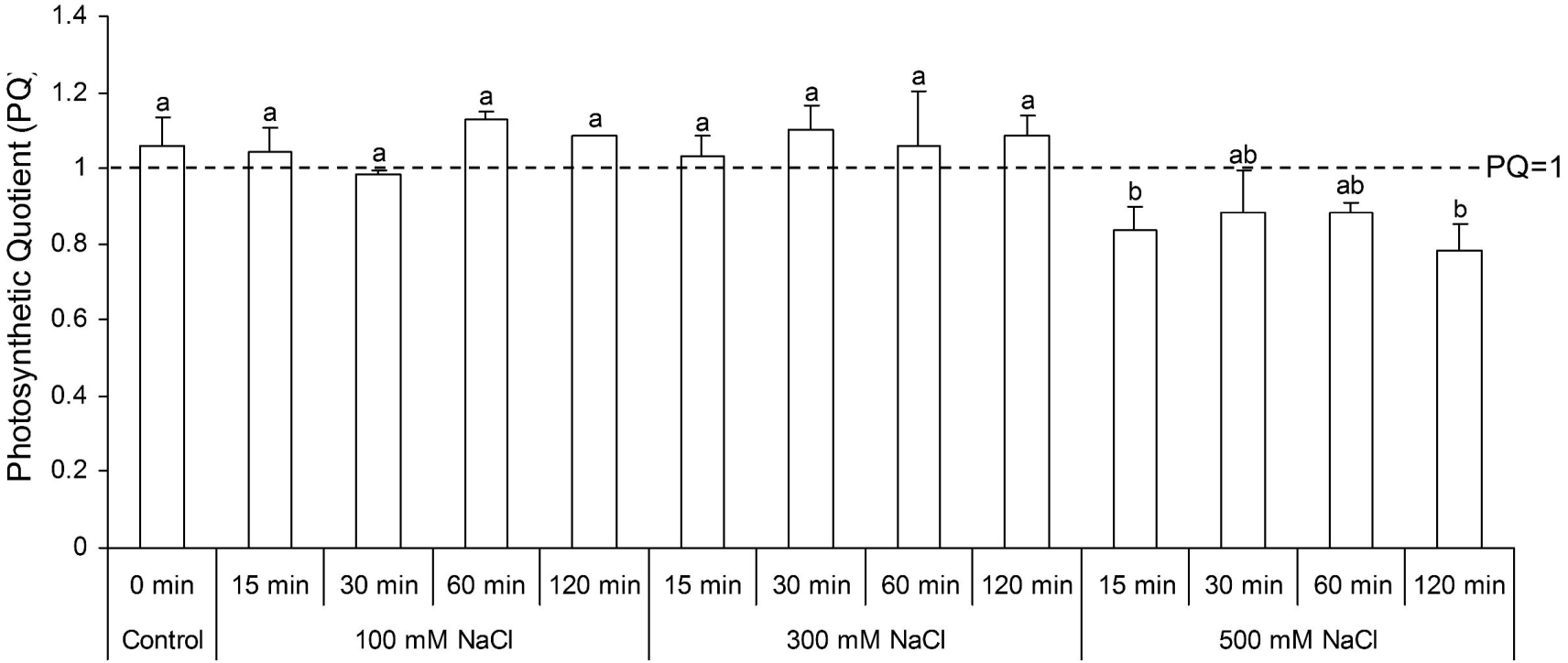


**Fig. 4.7** Photosynthetic quotients (PQ) of mangrove *R. mucronata* leaves subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Conditions of the medium was temperature $25^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. The standard error is shown above each bar. Values that are significantly different in the control and same NaCl concentrations ($P < 0.05$; Tukey HSD test) are indicated by different letters.

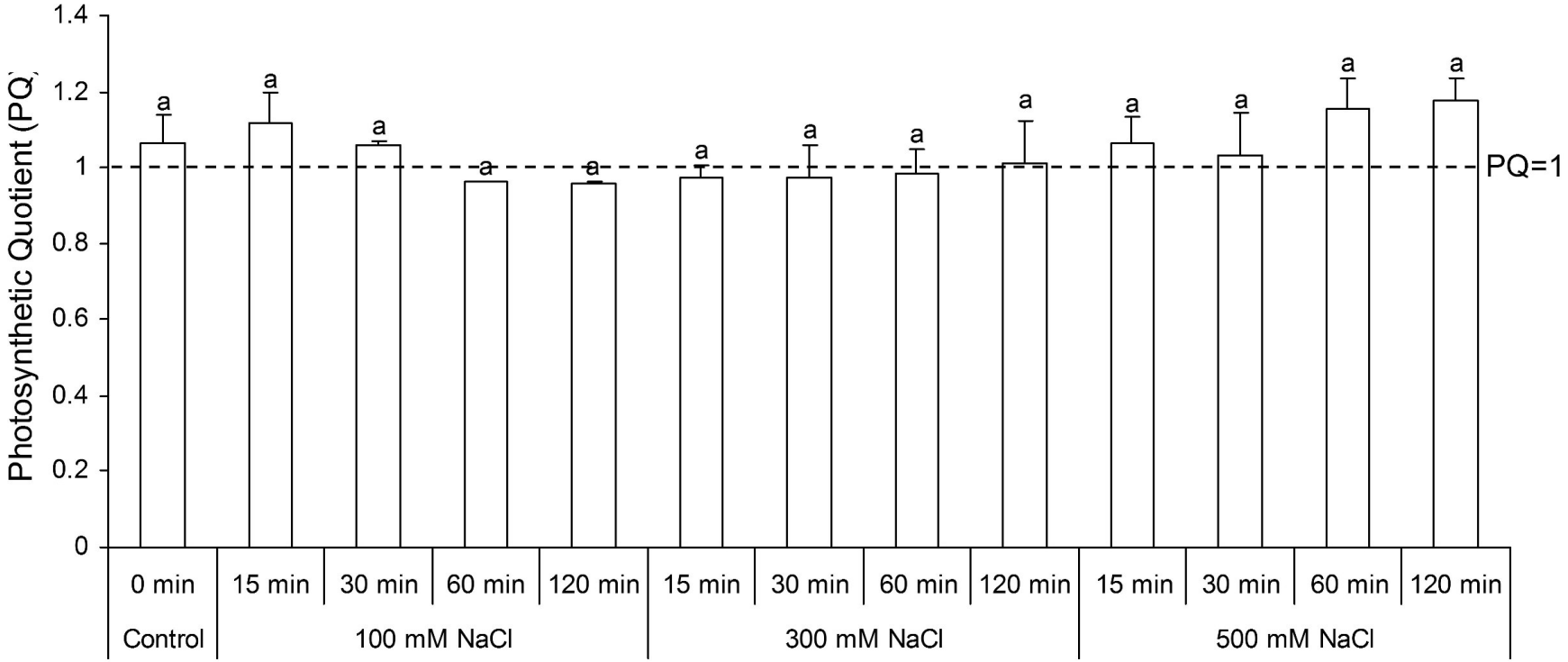


**Fig. 4.8** Photosynthetic quotients (PQ) of mangrove *A. marina* leaves subjected to variation of soaking periods and NaCl concentrations. Control leaves were not soaked (0 min) and no NaCl added. Conditions of the medium was temperature $25^0$C, Buffer pH 7.5, $NaHCO_3$ 20 mM. The standard error is shown above each bar. Values that are significantly different in the control and same NaCl concentrations ($P < 0.05$; Tukey HSD test) are indicated by different letters.

## 4. Discussion

Understanding potential photosynthetic performances of mangroves to salinity, flooding, and light were important contributions for diagnosing successful mangrove within tropic intertidal zone, managed or natural. This information will hopefully be helpful for explanations for mangrove distributional patterns, or ‘‘zonation’’.

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 Crithcley, 1982).

Under control condition, without salinity and soaking conditions, the photosynthetic rate-light performance of three mangroves stress under light saturation represented that *R. mucronata* > *B. gymnorrhiza* > *A. marina* (Figs 4.1-4.3). In natural mangrove forest of Hinchinbrook Channel, Australia, Clough (1998) has shown that response of net photosynthesis to light flux density of *Rhizophora spp* was higher than *B. gymnorrhiza.* In other side, Kawamitsu *et al*. (2003[b]) found that the leaf photosynthetic rate of *B. gymnorrhiza* was lower than *A. marina*. In this study, it was found that the maximum photosynthetic capacity of these three mangroves changed while subjected to salinity and soaking conditions with their specific responses.

In *B. gymnorrhiza* seedlings, it was obvious that all combinations of salinity and soaking stressed the leaf photosynthetic rate-light response more than other mangrove species (Fig. 4.1). It was important to note that low photosynthetic rate

and growth had consequences for mangrove light relations (Krauss *et al*., 2008; Ulqodry *et al*., 2014; Chapter 2), especially while interacted with other stressor like salinity (Ball, 2002; Lopez-Hoffman *et al*., 2007). The negative impacts of salinity on leaf-level carbon gain should be predominant at higher light levels, due to photosynthesis is limited by conductance during high light (Lambers *et al*., 1998). The significance decreasing of photosynthetic $O_2$ evolution and $CO_2$ uptake at high salinity probably related with low initial slope in *B. gymnorrhiza* leaves (Fig 4.5A). The quantum yield and quantum efficiency of photosystem II (PSII) can be estimated as the initial slope of the response photosynthetic rate to light intensity (Leverenz *et al*., 1990; Akhkha, 2010). The decrease in quantum yield suggested a reduction in the potential of PSII efficiency in mangrove (Tuffers *et al*., 2001). In other side, it is also often observed that photoinhibition results in a large decrease in the quantum yield in intact leaves (Björkman *et al*., 1988; Oquist and Malmberg, 1989; Leverenz *et al*., 1990). Photoinhibition is characterized by a light-dependent reduction in the fundamental quantum yield of photosynthesis and a loss of photosystem II (PSII) activity (Osmond, 1994). Björkman *et al*. (1988) observed that combination of saline environment and the potential for high radiation levels made avoiding photoinhibition a particular challenge for mangroves. Furthermore, photosynthetic-light respons was also lower in soaking than non-soaking conditions for all salinity levels, indicating that flooding were stressful to *B. gymnorrhiza* seedlings. Flooding created an overall reduced photosynthetic capacity for plant seedlings by prompting a reallocation of leaf and whole-seedling biomass (Krauss *et al*., 2008). The negative effects of flooding on photosynthesis from the leaf level to the plant level can lead to a low growth rate in flooded plants (Striker, 2012).

In contrast with *B. gymnorrhiza*, there was no negative effects of soaking conditions on photosynthetic rate-light response at low and middle salinity in *R. mucronata* (Fig 4.2). These results are in agreement with those obtained by Cardona-Olarte *et al*. (2006), which showed that no significance change of primary productivity of mangrove *Rhizophora* under low to mid stress by soaking and salinity. Furthermore, Hoppe-Speera *et al*. (2006) also found that seedlings growth of *R. mucronata* performed best in the moderate inundation and salinity, where high stomatal conductance was observed.

Similar with *R. mucronata*, little divergence existed among assimilation–light response curves by low-mid salinity for *A. marina* leaves subjected to different soaking conditions (Fig 4.3). These results seem to be consistent with Kawamitsu *et al*., (2003[a]) which found that the photosynthesis is not affected even when seedling plants always experiences flooding and the lower leaf of *A. marina*, which are submerged everyday. He *et al.* (2007) pointed out that in mangrove seedlings, flooding stressed on the leaf more than other organs. However, the mangrove filters seawater at the root system, allowing only fresh water to move to the above-round plant, consequently protecting the photosynthetic apparatus in the leaf (Kawamitsu *et al*., 2003[a]). Furthermore, the high initial slope while subjected to soaking periods and high salinity (Fig 4.5C) indicated that high PSII quantum efficiency, therefore, support the data in demonstrating a high degree of maximum photosynthetic rate in the leaves of *A. marina*.

Furthermore, photosynthetic performance of three mangrove species on soaking and salinity conditions based on simultaneous rate of $O_2$ evolution and $CO_2$

uptake is useful to clarify the PQ values for all treatments (Figs 4.6 – 4.8). The PQ value reflects the relative amounts of the major macromolecules (polysaccharides, proteins, lipids, and nucleic acids) synthesized by photosynthetic organisms (JGOF and SCOR, 2002). Theoretical PQ values typically range from 1.0–1.3 (Rosenberg *et al*., 1995) or up to 1.4 (Laws, 1991), whereas for new and recycled production are estimated to be 1.4 and 1.1, respectively (Laws, 1991). The PQ values were excess of 1.2 indicating significant production of storage materials (i.e., fats or protein) (Chisholm, 1998). This result indicated that the highest PQ usually lower than 1.2 for all treatments (Figs 4.6 – 4.8) might more reflect the recycled production in relation with internal process of photosynthesis rather than the production of new storage compunds such as fats and proteins. Some physiological functions that synthesise and consume $O_2$ and $CO_2$ may vary in an intact leaf, particularly under photorespiration (Rosenberg *et al*., 1995), stress conditions (Wu *et al*., 2014) and other oxygenative functions (Taddei *et al*., 2008).

A PQ value equal to unity (PQ = 1.00) might assume that $O_2$ production and $CO_2$ uptake in PSII and Calvin cycle in balance condition and there is no photorespiration during leave photosynthesis. Oxygen is produced in the reaction center of the PSII protein complex (Board, 2013) and leads to the formation of ATP and NADPH (Oettmeier, 1992; Cantin *et al*., 2007). In similar pathways, the Calvin cycle absorp $CO_2$ (Schwender *et al*., 2004; Goffman *et al*., 2005) and uses ATP and NADPH to synthesize Ribulose-1,5-bisphosphate (RuBP) (Tezara *et al*., 1999). Difference characteristics and pathways of movement of $O_2$ and $CO_2$ might indicate that $O_2$ evolution under stress may not be dependent on Calvin cycle function (Tezara *et al*., 1999), or photorespiration has occurred during photosynthesis

(Rosenberg *et al*., 1995). I supposed when photorespiration was included during the gas exchange, the PQ value will be not unity. Activating of the photorespiration will reduce the $CO_2$ absorption that resulted PQ>1. Photorespiration affect PQ value while rubisco, which principally functions as carboxylase, is substituted by the oxygenase function (Taddei *et al*., 2008).

Maximum photosynthetic $O_2$ evolution and $CO_2$ uptake of *B. gymnorhiza* under saline soaking periods usually lower than control (non saline soaking periods) (Fig 4.4A). This result was in line with the PQ values of *B. gymnorrhiza* always less than 1 under both mid and high salinity condition (Fig 6). PQ values of less than unity were not expected in macrophyta (Rosenberg *et al*., 1995). Chisholm (1998) point out that a fall in the value of PQ might occur when photosynthesis was light-limited and relatively had low growth. In other side, photosynthetic performance of *R. mucronata* and *A. marina* under saline soaking conditions almost similar or better than control condition, except for *R. mucronata* under high salinity 500 mM (Figs 4.4B and 4.4C). This result suggested that *B. gymnorhiza* was more intolerant to saline soaking periods than other 2 species. Since *Bruguiera* do not accumulate and excrete salts like *Avicennia,* slight dehydration of the tissues could partially contribute to reducing its photosynthetic performance. The anatomical characteristics of *B. gymnorrhiza* had a relatively low tolerance to waterlogging at the seedling stage (Wang *et al*., 2007) and also risky to the oxidant damage due to waterlogging (Ye *et al*., 2003).

Under low and mid salinity, *R. mucronata* always showed the PQ > 1 while soaking periods increased (Fig 4.7). *Rhizophora* generally appears to be more

tolerant of salinity and waterlogging than *Bruguiera* (Naidoo, 1985). During this condition, *R. mucronata* also indicated the high photosynthetic performance, with photosynthetic $O_2$ evolution more than 13 μmol $m^{-2}$ $s^{-1}$ and $CO_2$ uptake more than 12 μmol $m^{-2}$ $s^{-1}$ (Fig 4.4B). Although *Rhizopora* is not a salt excluder like *Avicennia,* but under salinity condition, their water use efficiency was uniquely increased with decreasing leaf water potential, that useful in maintaining the high photosynthetic rate even under severe stress condition (Kawamitsu *et al*., 1995). *R. mucronata*, ''the intermediate gap phase mangrove species'' had a role as main plant in tropical coastal area and produced high leaf litter (Wang'ondu and Virginia, 2010; Ulqodry *et al*., 2014; Chapter 2).

Regarding on the maximum photosynthetic rate and PQ values, the results in this study suppose that species differences in mangrove responses to soaking and salinity condition showed distinctions characteristic when PQ values < 1. In *B. gymnorrhiza* and *R. mucronata*, when PQ < 1, certainly $CO_2$ absorption reduced. It suggested the activation of photorespiration and other stresses. But in *A. marina*, $CO_2$ absorption rate was not changed significantly and still continues that indicated no activation of photorespiration and other stresses. Ball (1986) pointed out that there was no carbon losses via photorespiration in *A. marina* indicated the balancing between carboxylation and oxygen kinetics of rubisco.

This study indicated that among the 3 species, *A. marina* is best adapted to tolerate all salinities and soaking conditions and PQ values always around or higher than 1 (Figs 4.4C and 4.8). This study further support the idea of Naidoo (1985), *Avicennia* had adaptation capability to saline and waterlogging stress by maintaining

low stomatal resistance values, low tissue water potentials, high relative water content and high solute concentrations. In *R. mucronata* and *A. marina*, a better photosynthetic $O_2$ evolution and $CO_2$ uptake than *B. gymnorrhiza* on response to salinity and soaking condition, may reflects the high quantum yield in both species (Fig 4.5). The increment in quantum yield suggested an increase in the potential of PSII efficiency at mangrove (Krause and Winter, 1996; Tuffers *et al.,* 2001). The mangrove also protects the photosynthetic apparatus from destruction with its special function, even when water potential decreases remarkably (Kawamitsu *et al*., 2003[a]). Being the pioneer species in mangrove communities, *A. marina* adapted to broader habitats than *R. mucronata* and *B. gymnorrhiza*, although under control condition the photosynthetic performance of *A. marina* were inferior to *R. mucronata* and *B. gymnorrhiza*. The responses of mangroves to prolonged waterlogging and salinity indicate a remarkable adaptation to an intertidal environment (Naidoo, 1985). In contrast with other species, increasing of soaking periods under high salinity, had a positive effect on photosynthetic $O_2$ evolution and $CO_2$ uptake for *A. marina* (Figs 4.4.C and 4.8).

One potential cause of mangrove zonation is the differential ability of propagules to establish at different soaking condition (Kathiresan and Bingham, 2001). The result that indicated the photosynthetic performance and PQ values of *A. marina* > *R. mucronata* > *B. gymnorrhiza* by increasing of saline and soaking periods seem to be consistent with their zonation in natural habitat. In the West Indonesia, White *et al.* (1989), Whitten *et al.* (2000) and Suwignyo *et al.* (2012) identified that mangrove showed a characteristic zonation as characteristic : (1) *B. gymnorrhiza*, establish commonly on backside land area; (2) *R. mucronata*, occupy

dominantly in intermediate zone at the mid-tidal level; and (3) *A. marina,* the mangrove pioneer species, growth commonly in low intertidal swamps. It is clear that mangrove species respond differently to different salinity and soaking periods.

The next step is important to clarify the adapting mechanism of *A. marina* to keep the best photosynthetic performance under high salinity and how its performance under long soaking condition.

# CHAPTER 5

## General discussion

Understanding potential photosynthetic performances of mangroves to light, salinity and flooding were important contributions for diagnosing successful mangrove within tropic intertidal zone, managed or natural. This information will be helpful for an explanation of mangrove productivity, adaptation mechanism, their distributional patterns or ‘‘zonation’’, and also to develop the appropriate strategies to sustain mangrove rehabilitation.

In relation with mangrove rehabilitation, *R. mucronata* had a role as main plant in the reforestation thinned site in tropical coastal area (Srivastava *et al*. 1988) and produced more leaf litter than the reforestation unthinned and natural sites (Wang’ondu and Virginia 2010). 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.

Mangrove photosynthetic performance may vary with many factors, especially light intensity, salinity, flooding, and species composition (Alongi, 2009). The first study is started by investigating the seasonal photosynthetic responses and chlorophyll fluorescence in mangrove seedlings under shade regimes (Chapter 2). This study is useful to find the most suitable shading level during nurse phase of mangrove upon reforestation and cultivation, and also to clarify the zonation of *R. mucronata* as response to shade regimes. The second study improved the method of simultaneous determination of photosynthetic $O_2$ evolution and $CO_2$ uptake of

mangrove leaves under aqueous condition (Chapter 3). The substantial result from simultaneous measurement of $O_2$ evolution and $CO_2$ uptake is the ability to explore the photosynthetic quotient (PQ) values of mangrove leaves under aqueous conditions that will be used in the third study. The third study (Chapter 4) focused on investigation of the combined effect of salinity and soaking periods to mangrove zonation and productivity based on photosynthetic response from different mangrove species (*B. gymnorrhiza*, *R. mucronata*, and *A. marina*).

Mangroves belong to the $C_3$ plants, and 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 *R. mucronata,* "the intermediate gap-phase mangrove species", has been investigated seasonally under full sunlight (HL), 50% shading (ML) and 80% shading (LL) conditions (Chapter 2). Significant increases in total chlorophyll caused raising in $CO_2$ exchange were due to increased photosynthetic rate (Evans, 1989). 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 HL and ML had higher $P_N$ than LL leaves while PAR increasing. This study found that under light saturating conditions, $g_{max}$ and $E_{max}$ showed similar trends, they are LL<ML<HL respectively (Fig 2.5, Table 2.1). It described that the $P_{max}$ of *R. mucronata* seedlings were 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 (Xuan *et al*., 2011), and among of those factors, stomatal conductance is the most prominent (Putra *et al*., 2012).

Sharkey (1985) pointed out that the rates of photosynthesis were a function of both the stomata responses to allow $CO_2$ 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 *in vivo* assessment of biochemical components of leaf photosynthesis, such as ribulose-biphosphate carboxylase (rubisco) activity (Caemmerer and Farquhar, 1981). Furthermore, maximum photosynthetic rate responses to $C_i$ is beneficial to indicate the capacity or potential of leaf photosynthesis. As shown in Fig 2.8, the similar seasonal pattern of P' and $P_{max\text{-}Ci}$ 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, the results of this study showed that seasonal variation of temperature significantly affected P' and $P_{max\text{-}Ci}$. This result is in agreement with that of Campbell *et al*. (2005) whose findings showed 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 $P_{max\text{-}Ci}$ 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.

In relation with chlorophyll fluorescence, the regular value 0.75 - 0.85 of Fv/Fm ratios have been considered normal for unstressed plants (Hunt, 2003), and decline of Fv/Fm 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 2.11), 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). Furthermore, chronic photoinhibition of HL and ML leaves might cause decolouring of photosynthetic pigments such as chlorophyll and carotenoids (Powles, 1984; Takahashi *et al*., 2002). In contrast, it was found that LL plants sustained low susceptibility for photoinhibition. In the present study, although Fv/Fm of LL leaves declined during cold months, but the values were always higher than 0.75 (Fig 2.11) 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 under light (Alves *et al*., 2002). Although the mechanism is not clear, it was 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.

The study in Chapter 2 explored the photosynthetic performance of *R. mucronata* leaves using the gas exchange method. This method had a high precision and was rapid (Moore *et al*., 1973; Sobrado, 2005; Okimoto *et al*., 2007) but was limited under aqueous conditions as the Infra-Red Gas Analyser is sensitive to water immersion (Gevaert *et al*., 2011). In other side, the simultaneous estimation of $O_2$ and $CO_2$ has been done using isotope-Gas Chromatography-Mass Spectrometry (GC-

MS) with $^{13}CO_2$ and $^{18}O_2$ (Isobe *et al*., 2011). However, the method is unpopular because the equipment is very expensive (Sipior *et al*., 1996). The ability to measure photosynthetic rate under aqueous conditions, especially measurement of $O_2$ evolution and $CO_2$ uptake simultaneously, will be a useful mechanism to improve the studies of flooding and salinity effect on mangrove photosynthesis.

This study began to improve the method by comparing the most suitable leaf shape that resulted in highest $O_2$ evolution and $CO_2$ uptake, and also verified the suitable conditions of leaf photosynthetic rates by assessing pH levels and $NaHCO_3$ concentrations (Chapter 3). The result showed that *R. mucronata* leaf sample in sliced shape had higher $O_2$ evolution and $CO_2$ uptake rates compared with the chip shape. It suggests that slicing the leaf tissues facilitates the increasing of gas exchange across the boundary layer at the tissue surface (Brown, 1998). This eliminates the effect of stomatal resistance for $CO_2$ diffusion, and free $CO_2$ molecules or $HCO_3^-$ ions may penetrate more easily into the tissue of the leaf slice, resulting in a higher photosynthetic rate (Ishii *et al*., 1977).

Dissolved carbon dioxide in water occurs in three inorganic forms, free aqueous carbon dioxide (free $CO_2$), bicarbonate ($HCO_3^-$) and carbonate ions ($CO_3^{2-}$). If the equilibrium is affected by a change in pH, this could potentially influence $P_N$ (Riebesell *et al*., 2007). The $P_N$ is higher under intermediate pH values of 7.0–7.5 compared with low and high pH also demonstrated that the main carbon utilised as the substrate for *R. mucronata* leaf photosynthesis was free $CO_2$ molecules rather than bicarbonate. Almost all terrestrial plants use only free $CO_2$ for photosynthesis, however, many seaweeds or macroalgae use both free $CO_2$ and external bicarbonate

in water as a source of carbon for photosynthesis (Kawamitsu and Boyer, 1999; Pierini and Thomaz, 2004).

The important result from the second study is the ability to explore the photosynthetic quotient (PQ) values of *R. mucronata* leaves under aqueous conditions that can be described as;

$$PQ = \frac{O_2\ evolution}{CO_2\ uptake}$$

The PQ values of *R. mucronata* leaves in different pH and $NaHCO_3$ concentrations ranged within 1.04–1.28 with no significant difference among them. Stoichiometrically, a PQ value equal to unity which means PQ = 1.00 (Rosenberg *et al*., 1995). If this simple photosynthetic physiology was replaced by an ecological summation of protoplasm production, including carbohydrates, protein, lipids, and nucleic acids, then the theoretical PQ would be higher (Williams and Robertson, 1991). Theoretical PQ values typically range from 1.0 to 1.3 (Rosenberg *et al*., 1995), or up to 1.4 (Laws, 1991). This results also suggested that the simultaneous measurement of $O_2$ evolution and $CO_2$ uptake by using a Clark oxygen electrode type polarographic sensor and 'pCO2 mini' optodes sensor provided a simple, stable and precise measurement of net PQ under aqueous conditions.

Furthermore, the light saturation points for all $P_N$ measurements ($CO_2$ exchange in air, $O_2$ evolution under aqueous condition and $CO_2$ uptake under aqueous condition) were similar at PAR levels around 500–1,000 μmol photons $m^{-2}$ $s^{-1}$. All experiments produced comparable results with similar $P_{max}$ values of 13.37, 13.11 and 12.31 μmol $m^{-2}$ $s^{-1}$ for $CO_2$ exchange in air, $O_2$ evolution under aqueous conditions and $CO_2$ uptake under aqueous conditions, respectively. The $P_{max}$ value

and daily period of irradiance when plants were in the water and air would be useful as an indicator of primary production (Zimmerman *et al*., 1994). The similar $P_{max}$ values suggested that all treatments resulted in a high capacity to adjust the photosynthetic apparatus under light saturation conditions.

Based on the results of the second study (Chapter 3), the third study has investigated the photosynthetic $O_2$ evolution and $CO_2$ uptake responses of NaCl concentrations and soaking periods from different mangrove species (*Bruguiera gymnorrhiza*, *Rhizophora mucronata*, and *Avicennia marina*) (Chapter 4). In *B. gymnorrhiza* seedlings, it was obvious that all combinations of salinity and soaking stressed the leaf photosynthetic rate-light response more than other mangrove species (Fig. 4.1). The significant decreasing of photosynthetic $O_2$ evolution and $CO_2$ uptake at high salinity probably related with low initial slope in *B. gymnorrhiza* leaves (Fig 4.5A). The quantum yield and quantum efficiency of photosystem II (PSII) can be estimated as the initial slope of the response photosynthetic rate to light intensity (Leverenz *et al*., 1990; Akhkha, 2010). The decrease in quantum yield suggested a reduction in the potential of PSII efficiency in mangrove (Tuffers *et al*., 2001).

In contrast with *B. gymnorrhiza*, there was no negative effects of soaking conditions on photosynthetic rate-light response at low and middle salinity in *R. mucronata* (Fig 4.2). Hoppe-Speera *et al*. (2006) also found that seedlings growth of *R. mucronata* performed best in the moderate inundation and salinity, where high stomatal conductance was observed. Similar with *R. mucronata*, little divergence existed among assimilation–light response curves by low-mid salinity for *A. marina* leaves subjected to different soaking conditions (Fig 4.3). These results seem to be consistent with Kawamitsu *et al*. (2003[a]) which found that the photosynthesis is not

affected even when seedling plants always experiences flooding and the lower leaf of *A. marina*, which are submerged everyday. The high initial slope of *A. marina* while subjected to soaking periods and high salinity (Fig 4.5C) indicated that high PSII quantum efficiency, therefore, support the data in demonstrating a high degree of maximum photosynthetic rate in the leaves of *A. marina*.

The PQ values were excess of 1.2 indicating significant production of storage materials (*i.e.*, fats or protein) (Chisholm, 1998). This result indicated that the highest PQ usually lower than 1.2 for all treatments (Figs 4.6 – 4.8) might more reflect the recycled production in relation with internal process of photosynthesis rather than the production of new storage compunds such as fats and proteins. Some physiological functions that synthesise and consume $O_2$ and $CO_2$ may vary in an intact leaf, particularly under photorespiration (Rosenberg *et al*., 1995), stress conditions (Wu *et al*., 2014) and other oxygenative functions (Taddei *et al*., 2008).

A PQ value equal to unity (PQ = 1.00) might assume that $O_2$ production and $CO_2$ uptake in PSII and Calvin cycle in balance condition and there is no photorespiration during leave photosynthesis. Oxygen is produced in the reaction centre of the PSII protein complex (Board, 2013) and leads to the formation of ATP and NADPH (Oettmeier, 1992; Cantin *et al*., 2007). In similar pathways, the Calvin cycle fix $CO_2$ (Schwender *et al*., 2004; Goffman *et al*., 2005) and uses ATP and NADPH to synthesize RuBP (Tezara *et al*., 1999). Difference characteristics and pathways of movement of $O_2$ and $CO_2$ might indicate that $O_2$ evolution under stress may not be dependent on Calvin cycle function (Tezara *et al*., 1999), or photorespiration has occurred during photosynthesis (Rosenberg *et al*., 1995). It was supposed that the PQ value will be not unity when photorespiration was included

during the gas exchange. Activating of the photorespiration will reduce the $CO_2$ absorption that resulted PQ>1. Photorespiration affect PQ value while rubisco, which principally functions as carboxylase, is substituted by the oxygenase function (Taddei *et al*., 2008).

Maximum photosynthetic $O_2$ evolution and $CO_2$ uptake of *B. gymnorhiza* under saline soaking periods were usually lower than control (non saline soaking periods) (Fig 4.4A). This result was in line with the PQ values of *B. gymnorrhiza* always less than 1 under both mid and high salinity condition (Fig 6). PQ values of less than unity were not expected in macrophyta (Rosenberg *et al*., 1995). Chisholm (1998) point out that a fall in the value of PQ might occur when photosynthesis was limited by light and relatively had low growth. In other side, photosynthetic performance of *R. mucronata* and *A. marina* under saline soaking conditions almost similar or better than control condition, except for *R. mucronata* under high salinity (Figs 4.4B and 4.4C). This result suggested that *B. gymnorhiza* was more intolerant to saline soaking periods than other 2 species. Since *Bruguiera* do not accumulate and excrete salts like *Avicennia,* slight dehydration of the tissues could partially contribute to reducing its photosynthetic performance. The anatomical characteristics of *B. gymnorrhiza* had a relatively low tolerance to waterlogging at the seedling stage (Wang *et al*., 2007) and also risky to the oxidant damage due to waterlogging (Ye *et al*., 2003).

Under low and mid salinity, *R. mucronata* always showed the PQ > 1 while soaking periods increased (Fig 4.7). *Rhizophora* generally appears to be more tolerant of salinity and waterlogging than *Bruguiera* (Naidoo, 1985). During this condition, *R. mucronata* also indicated the high photosynthetic performance, with photosynthetic $O_2$ evolution more than 13 μmol $m^{-2}$ $s^{-1}$ and $CO_2$ uptake more than 12

μmol $m^{-2}$ $s^{-1}$ (Fig 4.4B). Although *Rhizopora* is not a salt excluder like *Avicennia,* but under salinity condition, their water use efficiency was uniquely increased with decreasing leaf water potential, that useful in maintaining the high photosynthetic rate even under severe stress condition (Kawamitsu *et al*., 1995).

This study indicated that among of the 3 species, *A. marina* is best adapted to tolerate all salinities and soaking conditions and PQ values always around or higher than 1 (Figs 4.4C and 4.8). This study further support the idea of Naidoo (1985), *Avicennia* had adaptation capability to saline and waterlogging stress by maintaining low stomatal resistance values, low tissue water potentials, high relative water content and high solute concentrations. In *R. mucronata* and *A. marina*, a better photosynthetic $O_2$ evolution and $CO_2$ uptake than *B. gymnorrhiza* on response to salinity and soaking condition, may reflects the high quantum yield in both species (Fig 4.5). The mangrove also protects the photosynthetic apparatus from destruction with its special function, even when water potential decreases remarkably (Kawamitsu *et al*., 2003[a]).

Vegetational zonation of the mangroves, a frequently conspicuous feature, has long attracted scientific interest (Bunt, 1996). Different mangrove species tend to occupy specific zone or specific habitat zonation (Bunt, 1996; Youssef and Saenger, 1999). Species differences in mangrove responses to the interactive effects of some stress conditions might explain important differences in mangrove forest structure (Krauss *et al*., 2008). ). In relation with photosynthetic-light respons competition, previous studies have investigated that gas exchange and chlorophyll fluorescence characteristics of mangrove *Avicennia marina* (growth well in sea side area) showed typical of sun leaves (Ball and Crithcley, 1982). On the other hand, *Bruguiera*

(growth well in land side area) responded favorably to short burst of sunlight at low light level and relatively shade tolerant species (Krauss and Allen, 2003). However, there is no detail information about photosynthetic light response of *R. mucronata*, "the intermediate gap-phase mangrove species". The result showed that *R. mucronata* grown under LL condition had lower $P_N$ than HL and ML while PAR increasing. Lower rates of $g_{max}$ for LL leaves probably restricted the maximum photosynthetic rate, that similarly as shown at "the shade tolerant mangrove species", *Bruguiera sexangula* (Krauss and Allen, 2003). The important finding of this study showed that *R. mucronata* grown under ML condition provided the effectiveness to obtain such high carbon fixation capacity (Chapter 2). It was indicated by the ability of ML leaves to achieve high $P_{max}$ in lower $g_{max}$ and $E_{max}$ compared with HL leaves (Fig 2.5). The ML effectiveness in $g_s$ controlling also provide chance to conserve water in better level. It will be useful while ML seedlings adapt with saline condition. This result might also indicate that *R. mucronata* as the intermediate zone mangrove species prefer more in moderate shade condition rather than full sunny like *A. marina*, or deep shade condition like *B. gymnorrhiza*.

The mangrove zonation patterns are also might correlate with the tidal immersion and the salinity of water (Ball, 1988). This finding showed that all combinations of salinity and soaking stressed the leaf photosynthetic rate-light response in *B. gymnorrhiza* seedlings more than other mangrove species (Fig. 4.1, Chapter 4). Maximum photosynthetic-light respons was also lower in soaking than non-soaking conditions for all salinity levels, indicating that flooding were stressful to *B. gymnorrhiza* seedlings (Fig 4.4, Chapter 4). Flooding created an overall reduced photosynthetic capacity for plant seedlings by prompting a reallocation of

leaf and whole-seedling biomass (Krauss *et al*., 2008). The negative effects of flooding on photosynthesis from the leaf level to the plant level can lead to a low growth rate in flooded plants (Striker, 2012). In other side, photosynthetic performance of *R. mucronata* and *A. marina* under saline soaking conditions almost similar or better than control condition, except for *R. mucronata* under high salinity (Figs 4.4B and 4.4C). This result suggested that *B. gymnorhiza* was more intolerant to saline soaking periods than other 2 species. In addition, the initial slope of the response photosynthetic rate to light intensity from each species affected maximum photosynthetic rate significantly (Fig 4.4 and 4.5, Chapter 4). The increment in quantum yield suggested an increase in the potential of PSII efficiency at mangrove (Krause and Winter, 1996; Tuffers *et al.,* 2001). Regarding on the PQ values, it was suggested that species differences in mangrove responses to soaking and salinity conditions showed distinct characteristics when PQ values < 1. In *B. gymnorrhiza* and *R. mucronata*, when PQ < 1, certainly $CO_2$ absorption reduced. This result suggested the activation of photorespiration and other stresses. But in *A. marina*, $CO_2$ absorption rate was not changed significantly and still continues that indicated no activation of photorespiration and other stresses. Ball (1986) pointed out that there was no carbon losses via photorespiration in *A. marina* indicated the balancing between carboxylation and oxygen kinetics of rubisco. In contrast with other species, increasing of soaking periods under high salinity, had a positive effect on photosynthetic $O_2$ evolution and $CO_2$ uptake for *A. marina* (Figs 4.4.C and 4.8). Being the pioneer species in mangrove communities, *A. marina* adapted to broader habitats than *R. mucronata* and *B. gymnorrhiza*. The result that indicated the photosynthetic performance and PQ values of *A. marina* > *R. mucronata* > *B.*

*gymnorrhiza* by increasing of saline and soaking periods seem to be consistent with their zonation in natural habitat.

In recent decades, mangroves have been planted to counter the effect of degradation by human activities in coastal ecosystems of tropics and subtropics (Okimoto *et al*., 2007). The main purpose of mangrove forestation is not only restoration, but also an expansion of the mangrove area, because mangroves are widely regarded as a significant sink for atmospheric $CO_2$ (Clough, 1998). Among mangrove species, *R. mucronata* had a role as main plant in the reforestation thinned site in tropical coastal area (Srivastava *et al*., 1988) and produced more leaf litter than the reforestation unthinned and natural sites (Wang'ondu and Virginia, 2010). The role of *R. mucronata* in reforestation activity will become more effective and crucial while the most suitable thinning level could be identified. Mangrove density and tree-fall gaps create contrasting low- and high-shading areas. Seedlings adaptive capacity to shade regimes in relation to photosynthetic performances will contribute on the potential productivity of thinning activity. The finding in Chapter 2 indicated that photosynthetic performance of *R. mucronata* grown under moderate shading was higher than under deep shading. This result supported the using of *R. mucronata* in mangrove thinning activity as regards to gain high $CO_2$ fixation in line with wood production for local people. It means, mangrove reforestation could be managed by an appropriate thinning practice that reducing tree numbers along their growth without decreasing their function as Carboon pool.

In this study, it was concluded that seedling of *R. mucronata* grown under moderate shade condition provided better condition to obtain such carbon fixation capacity than deep shade condition. This result clarified the suitable shading level

during nurse phase of *R. mucronata* upon reforestation and cultivation. Simultaneous measurements of $O_2$ evolution and $CO_2$ uptake is useful to explore the PQ values of mangrove. Furthermore, *A. marina* could be as potential plant for mangrove rehabilitation and productivity in the future especially during increasing of sea level due to global warming due to its ability to gain high photosynthetic rate and PQ values under high salinity and flooding conditions.

Finally, the preference and tolerance of each mangrove species based on photosynthetic performance in relationship with their zonation and productivity were summarized in Fig 5.1. *B. gymnorrhiza* is characterized with the highest shade preference, but low tolerance to salinity and flooding, so this species was more suitable for reforestation activity with non-thinning system. In other side, *R. mucronata* has medium tolerance with the highest photosynthetic rate make this species as the most suitable for reforestation activity with thinning system. Furthermore, *A. marina* show the most tolerance species to salinity and flooding conditions, make it as potential plant for mangrove rehabilitation and productivity in the future especially during increasing of sea level due to global warming. These results are important in order to discover the potential functions from each mangrove species in mangrove reforestation activity.

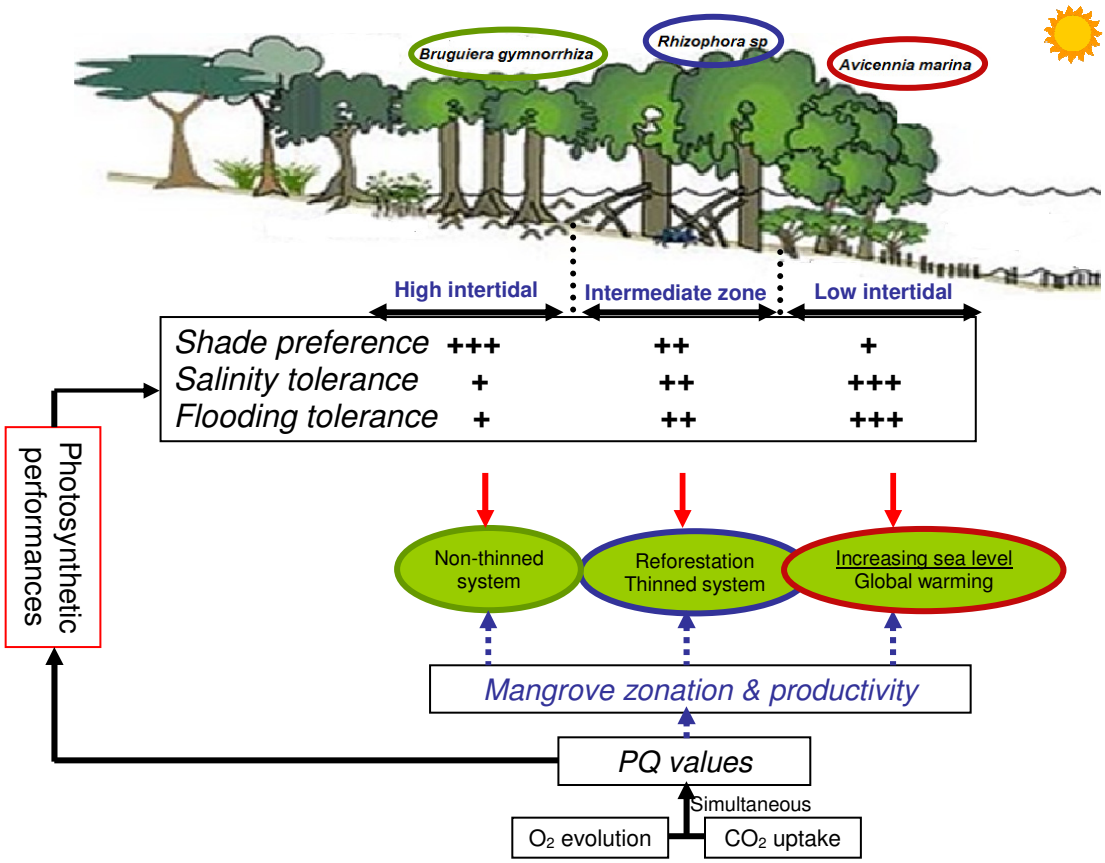


**Fig 5.1.** A diagram for describing the environmental condition preference and tolerance of each mangrove species based on photosynthetic performance in relationship with their zonation and productivity in order to discover their potential function in mangrove reforestation activity.

## Future studies

During investigations on the photosyntehtic responses and chlorophyll fluorescence of mangrove *Rhizophora mucronata* seedling under shade regimes, as shown in Chapter 2, it was found that although LL leaves photosynthetic rate is lower generally than HL and ML but never showed photoinhibition during the experiments (Fv/Fm ratio always > 7.5). 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 under light (Alves *et al*., 2002). However, the mechanism is not clear, these results just 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. The expression levels of several photosynthesis- and hormon-related genes were significantly affected by the light intensity (Majláth *et al*., 2012). Mangrove photosynthetic responses to various shade regimes and temperature may have an influence not only physiologically but also biochemical processes.

In order to improve the simultaneous measurement of $O_2$ evolution and $CO_2$ uptake under aqueous condition (Chapter 3), the method in this study worked well under light saturation compared with light limitation. At low light levels, the photosynthetic rate of $O_2$ evolution and $CO_2$ uptake under aqueous conditions was lower than the photosynthetic $CO_2$ exchange in air. This result is likely to be related to the reduction of low light utilisation while the leaf slices were rotated under aqueous conditions. Therefore, this study need to be improved the simultaneous

measurements of photosynthetic $O_2$ evolution and $CO_2$ uptake under aqueous conditions in low light conditions.

Furthermore, the third study in Chapter 4 indicated that in contrast with other mangrove species, increasing of soaking periods under high salinity, had a positive effect on photosynthetic $O_2$ evolution and $CO_2$ uptake for *A. marina.* Among three mangrove species, *A. marina* is best adapted to tolerate all salinities and soaking conditions and PQ values always around or higher than 1. The next step is important to clarify the adapting mechanism of *A. marina* to keep the best photosynthetic performance under high salinity and how its performance under long soaking condition.

Generally, these studies on mangrove photosynthetic performance and its characteristics based mainly on physiological mechanism. There are few reports on the combined effects of salinity and flooding in mangrove (Cardona-Olarte *et al*. 2006) especially related with protein expression-stress response. It has been reported that the protein components of stress-defense response such as salt stress protein "mangrin" is represented in mangrove *Bruguiera sexangula* (Yamada *et al*., 2002) and *Rhizophora mucronata* (Inomata *et al*., 2009) but available in difference molecule weight and pI (http://www.uniprot.org and http://web.expasy.org). The next study is important to elucidate the characteristic and role of mangrin and other specific proteins expression from diferrent mangrove zonation species. Understanding the physiological, biochemical and molecular mechanisms of the combined stress is necessary to develop the growth model and appropriate strategies to sustain plant production.

## Summary

Mangroves are the unique $C_3$ coastal plant living in specific habitat in the interface between land and sea, and show high productivity and have specific zonation. Mangrove provide a wide range of services in regard to shoreline erosion control, promotion of sustainable fisheries, and high carbon fixation capacity. Leaf $O_2$ evolution and $CO_2$ uptake are fundamental mechanisms that support oxygen and carbon ecosystems from the individual plant to the global scale. The main objective of this study is to investigate photosynthetic performance in mangrove leaves as regards their productivity and adaptability mechanisms. This information will be useful not only to explain mangrove productivity but also to elucidate the mangrove zonation and for diagnosing successful mangrove within tropic intertidal zone, managed or natural.

As regards light competition, gas exchange and chlorophyll fluorescence characteristics of mangrove *R. mucronata,* "the intermediate gap-phase mangrove species", has been investigated seasonally under full sunlight (HL), 50% shading (ML) and 80% shading (LL) conditions. The carboxylation efficiency significantly affected the seasonal change of the photosynthetic capacity. The photosynthetic rate ($P_N$) of *R. mucronata* seedlings under shade regimes, however, could not be attributed to variability in chlorophyll, Ci, ΦPSII, ETR or qP values but more to differences in carboxylation efficiency, maximum stomatal conductance ($g_{max}$), and maximum transpiration rate ($E_{max}$). HL and ML plants had higher $P_N$, $g_{max\ s}$ and $E_{max}$ than the LL ones. Nevertheless, LL leaves exhibited low photoinhibition susceptibility.

Traditional method for the gas exchange of photosynthesis has been assessment of either $O_2$ evolution or $CO_2$ uptake. In this study a liquid-phase $O_2$ electrode combined with $CO_2$ optodes was used to examine simultaneously photosynthesis in intact leaves of mangrove *Rhizophora mucronata* under aqueous condition. The photosynthetic rate in response to pH exhibited a similar pattern both for $O_2$ evolution and $CO_2$ uptake, and higher rates were associated with intermediate pH compared with low and high pH values. The similar maximum photosynthetic rates suggested that all measurements had a high capacity to adjust the photosynthetic apparatus under a light saturation condition.

Finally, in this study it was clarified the photosynthetic performance of different mangrove zonation species (*Avicennia marina*, *Rhizophora mucronata*, and *Bruguiera gymnorrhiza*) under a combination of salinity and NaCl soaking stress. Photosynthesis rate and photosynthetic quotient (PQ) for each mangrove seedlings showed different responses with increasing the soaking period and NaCl concentration. Among three mangrove species, photosynthetic performance in *B. gymnorrhiza* was decreased significantly as compared with other species. In other side, photosynthetic performance of *A. marina* was uniquely increased with increasing the soaking period and NaCl concentration. It showed that *A. marina* maintained the high photosynthetic rate even under the soaking condition. *R. mucronata* had an intermediate response to NaCl concentration during the soaking periods.

This study conclude that seedling of *R. mucronata* grown under moderate shade condition provided better condition to obtain such carbon fixation capacity than deep shade condition. This result is important to clarify the suitable shading level during

nurse phase of *R. mucronata* upon reforestation and cultivation. Furthermore, the simultaneous measurements of $O_2$ evolution and $CO_2$ uptake using a Clark oxygen electrode with $CO_2$ optode sensor provided a simple and stable measurement that useful to explore the PQ values of mangrove leaves under aqueous and saturated light conditions. Finally, based on the rank order of the photosynthetic performance and PQ values to saline and the soaking periods among these three mangrove species was, from most to least tolerant, *A. marina* > *R. mucronata* > *B. gymnorrhiza*. The result proposed that the ability of *A. marina* to gain high PQ values under high salinity and flooding conditions could be as potential plant for mangrove rehabilitation and productivity in the future especially during increasing of sea level due to global warming.

## Abstract in Japanese

マングローブは潮間帯に生育する $C_3$ 植物で、高い生産性と特異な帯状群落を形成し、海岸線保護や沿岸漁業において有意な資源であることが知られている。また、葉の光合成に由来する $O_2$ 放出と $CO_2$ 吸収は、個体レベルの生育から地球規模の環境問題にまでつながる基本的な植物の機能である。本研究では、マングローブ群落の示す特異な帯状構造と海水浸漬への適応について、光合成特性から検討を加えた。

帯状の群落を形成する要因として、その中間帯に分布するオオバヒルギ（*Rhizophora mucronata*）における光環境への適応特性に着目し、無遮光区(HL)、50％遮光区(ML)、80%遮光区(LL)における光合成の特性の季節変化をガス交換とクロロフィル蛍光特性を調査した。HL 及び ML 区の光合成速度（$P_N$)は、LL 区に比べ高く推移し、その原因は、クロロフィル含量、葉内 $CO_2$ 濃度（Ci）、 量子収率（ΦPSII）、 電子伝達速度（ETR）や光化学的消光特性（qP）によるものではなく、炭素固定能力や、気孔コンダクタンス（$g_{max}$）によることが明らかになった。また、LL 区では、低温季にける光合成の光阻害感受性が低下することも明らかになった。

光合成における $O_2$ 放出と $CO_2$ 吸収を同時測定する手法を、液相型電極を用いて開発した。液相の pH の変化に対し、$P_N$ は $O_2$ 放出と $CO_2$ 吸収の両方で同様の傾向を示し、低 pH 値や高 pH 値よりも pH7.0～7.5 で高い $P_N$ が観測された。なお、pH 反応及び基質である $NaHCO_3$ に対する反応共に $O_2$ 放出による $P_N$ の評価が有意ではないものの高くなる傾向を示した。

塩化ナトリュウム（NaCl）濃度と NaCl 水浸漬ストレスを組み合わせた条件下で、マングローブの帯状群落を代表する 3 種(*Avicennia marina*, *R. mucronata*, and *Bruguiera gymnorrhiza*)の光合成特性を検討した。*B. gymnorrhiza*（オヒルギ）の $P_N$ は他の種に較べ、NaCl 濃度の上昇及び浸漬時間の延長に伴い低下する程度が大きかった。*A. marina*（ヒ

ルギダマシ）の $P_N$ は、他種と異なり、浸漬時間の延長及び NaCl 濃度の上昇に伴い高くなるという特異的な $P_N$ 反応を示した。*R. mucronata* は中間的な反応を示した。つまり、海水濃度と海水浸漬時間に対する適応性については、*A. marina* > *R. mucronata* > *B. Gymnorrhiza* の順で低下し、その要因のひとつに光合成特性が関与することが明らかになった。

以上の結果は、帯状群落の中間に分布する *R. mucronata* 幼苗の光合成特性は、陽葉と陰葉が示す両方特性を併せ持ち、その特性をマングローブ植林の主要種である *R. mucronata* の育苗や間伐等による森林技術の開発に活用することが期待される。また、海水濃度と海水浸漬時間に対する光合成の適応性の種による違いは、マングローブ植林に際しての樹種の選定に有効な情報となる。さらに、液相型電極を用いた光合成の $O_2$ 放出と $CO_2$ 吸収の同時測定法は、マングローブに限らず他の植物の光合成のストレス反応を非破壊的に把握する簡便な手法として有効なものと考えられる。

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