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THESIS

PHOTOSYNTHESIS OF RUBBER (*Hevea Brasiliensis* Muell. Arg.) - EFFECT OF TEMPERATURE, LEAF AGE AND SEASON

BOONTHIDA KOSITSUP

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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The aim of this study was to assess effect of temperature, leaf age, and season on single leaf photosynthesis rates in rubber trees (*Hevea brasiliensis* Muell.Arg.). Net CO_2 assimilation rate (A) was measured in rubber saplings grown in a nursery and in growth chambers at 18 ˚C and 28 ˚C. The temperature response of A was measured from 9 to 45 °C and the data were fitted by an empirical model. The photosynthetic capacity (maximum carboxylation rate, V_{cmax}, and maximum electron transport rate, J_{max}) of plants acclimated to 18 ˚C and 28 ˚C were estimated by fitting a biochemical photosynthesis model to the CO_2 response curves (A/C_i curves) at 15, 22, 28, 32, 36 and 40 ˚C. The optimum temperature (T_{opt}) for A was lower in plants grown at lower temperature (18 ˚C) than plants grown at higher temperature (28 ˚C) in growth chambers. Net CO_2 assimilation at optimum temperature (A_{opt}), V_{cmax} and J_{max} at a reference temperature of 25 ˚C (V_{cmax25} and J_{max25}) as well as activation energy of V_{cmax} and J_{max} (E_{aV} and E_{aJ}) decreased at 18 °C growth temperature as compared to 28 °C growth temperature. The optimum temperature for V_{cmax} and J_{max} was not defined for certain value, but they were above 36 ˚C and not far from 40 ˚C. The average ratio of J_{max25}/V_{cmax25} was higher in plants grown at lower temperature (18 ˚C). Variations in A, V_{cmax} and J_{max} were partly explained by leaf nitrogen content. These results indicate that rubber leaves are able to change their photosynthetic characteristics in response to growth temperature.

Finally, leaf age and seasonal effects on photosynthesis were studied in 2-year-old rubber trees grown in rubber plantation. Maximum net CO_2 assimilation (A_{max}), V_{cmax25}, J_{max25}, and nitrogen use efficiency (A_{max}/N_a) decreased significantly with increasing leaf age. Lower temperature and drier season also decreased photosynthesis of 2-year-old rubber trees in the field. These results indicate that not only temperature, but also age and season-related effect must to be taken into account in photosynthetic study in rubber trees.

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Student's signature Thesis Advisor's signature

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Boonthida Kositsup
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LIST OF ABBREVIATIONS

A = Net CO_2 assimilation rate (µmol CO_2 $m^{-2}s^{-1}$)

A_c = RuBP carboxylation-limited photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)

A_j = RuBP regeneration-limited photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)

A_{max} = Light-saturated net photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)

C_i = Partial pressure of CO_2 in the intercellular air space (Pa)

E_a = Activation energy of the R_d, τ, K_c and K_o (J mol^{-1})

E_{aJ} = Activation energy for J_{max} (J mol^{-1})

E_{aV} = Activation energy for V_{cmax} (J mol^{-1})

g_i = Internal conductance (mmol CO2 m^2 s^{-1})

g_s = Stomatal conductance (mmol H_2O m^2 s^{-1})

J = Rate of electron transport (µmol $m^{-2}s^{-1}$)

J_{max} = Maximum electron transport rate (µmol $m^{-2}s^{-1}$)

K_c = Michaelis constant of Rubisco for carboxylation (Pa CO_2)

K_m = Maximum conductivity (mmol m s^{-1} MPa^{-1})

K_o = Michaelis constant of Rubisco for oxygenation (Pa O_2)

LMA = Leaf mass per area (g m^{-2})

N_a = Nitrogen content basis on area (g m^{-2})

N_m = Nitrogen content basis on mass (%)

O = Partial pressure of O_2 in the intercellular air space (Pa)

P_a = Atmospheric pressure (Pa)

PPFD = Photosynthetic photon flux density (µmol $m^{-2}s^{-1}$)

Q = Incident photon flux density (µmol.m^{-2}.s^{-1})

R_{gas} = Gas constant (8.3143 J K^{-1} mol^{-1})

R_d = Rate of CO_2 evolution in light that results processes other than photorespiration (µmol CO_2 $m^{-2}s^{-1}$)

SPAD = Leaf greenness (SPAD unit)

T_l = Leaf temperature (K)

T_{opt} = Optimal temperature

LIST OF ABBREVIATIONS (Continued)

T_{growth} = Growth temperature

V_{cmax} = Maximum carboxylation rate (μmol CO_2 $m^{-2}s^{-1}$)

α = Apparent efficiency of light energy conversion on an incident light basis (mol electrons per mol photons)

τ = Specificity factor for Rubisco

PHOTOSYNTHESIS OF RUBBER (*Hevea brasiliensis* Muell. Arg.) - EFFECT OF TEMPERATURE, LEAF AGE AND SEASON

INTRODUCTION

Rubber (*Hevea brasiliensis* Muell. Arg.) is a major tropical crop exploited by tapping for latex production, planted mainly in South-East Asia. However, rubber tree plantations are currently expanding to non-traditional areas such as Northern and Northeastern Thailand, Yunnan in China or Mato Grosso in Brazil, where trees encounter more extreme (cold and hot) temperatures. Environmental conditions such as temperature influence the growth of rubber and latex yield (Raj *et al*., 2005, and references therein). The latex production rate in the latex vessels has been related to the supply of sucrose in the adjacent sieve tubes. Thus, it is apparent that economic yield in rubber crop is closely linked to net CO_2 assimilation rate which is the primary source of sucrose. This possible link has promoted examination of the potential of selecting higher yield by screening for higher photosynthetic capacity (Nugawela *et al*., 1995c). However, little is known about net CO_2 assimilation rate of rubber leaves in a wide range of temperatures, covering both hot and cold possible temperature in new plantation areas.

Temperature is an important factor affecting biochemical processes of photosynthesis inside a leaf. Understanding the temperature response of photosynthesis is important for the modellers to predict the response of plants to changes in climatic conditions. Such changes could occur in the future as the average global temperature is predicted to increase by approximately 2-4 °C for the next 100 years (IPCC, 2007). Plants can also face unusual climatic conditions when they are introduced in a new area.

It is well known that photosynthesis can change in response to environmental condition, e.g. temperature and nitrogen availability (Berry and Björkman, 1980; Dreyer *et al*., 2001; Kattge and Knorr, 2007). However, leaf age also affects on photosynthetic productivity. Many studies on temperature response of photosynthetic capacity have been assessing on young fully expanded leaves, ignoring leaf-age effect (Hikosaka *et al*., 1999; Yamori *et a*l., 2005, 2006; Hikosaka *et al*., 2007). Kitajima *et al*. (2002) found that photosynthetic rates and nitrogen use efficiency in tropical pioneer tree species, *Cecropia longipes* and *Urera caracasana*, were negatively correlated with leaf age. The decline in photosynthesis can be attributed to the remobilization of nitrogen (N) from older leaves to younger leaves or N allocation within leaves (Han *et al*., 2008). Schoettle and Smith (1998) showed that old leaves had a lower $A_{\max}$ than young leaves in a similar daily irradiance regime. Niinemets *et al*. (2005) showed that photosynthesis and photosynthetic nitrogen use efficiency decreased with increasing leaf age; and older leaves had lower V_{cmax} and $J_{\max}$. Onoda *et al*. (2005b) found that $J_{\max}/V_{\mathrm{cmax}}$ ratio decreased from summer to autumn may be a result of leaf senescence rather than an environmental acclimation.

Sangsing (2004) found the relationship between V_{cmax} and leaf temperature of two rubber clones. V_{cmax} increased as leaf temperature increased from 27 to 34 °C. However, the range of leaf temperature of this study was narrow. Moreover, data necessary to assess temperature acclimation of photosynthesis using Farquhar’s model (Farquhar *et al*., 1980) in rubber trees are lacking. As a whole, such data remain scarce in tropical trees (Kattge and Knorr, 2007). In addition, most studies on temperature response and acclimation of photosynthesis have been conducted on young fully expanded leaves, ignoring the effect of leaf age (Han *et al*., 2008). Thus the present study has been focused on temperature response and acclimation of photosynthesis in a wide range of leaf temperature and the effect of leaf age on photosynthesis of rubber grown in the field.

OBJECTIVES

The main objectives of this study are to understand effect of temperature, leaf age, and season on single leaf photosynthesis rates in rubber trees (*Hevea brasiliensis* Muell.Arg.). The following questions are addressed in the present study:

1) What is the temperature response of net CO_2 assimilation (A) of rubber grown in a nursery in Thailand and in a growth chamber at 28 °C ?

2) Does photosynthetic capacity as described by the model parameters (V_{cmax}, J_{max}) at a reference temperature of 25 °C change after an acclimation to different growth temperatures?

3) Does the temperature response of A and of V_{cmax} and J_{max} as described by optimum temperature and activation energy change with growth temperatures?

4) Does leaf age affect photosynthesis of rubber trees?

5) How does photosynthesis of field-grown rubber trees change with season?

These questions are answered with: 1) data from an experiment with saplings of rubber trees grown in a nursery at ambient temperature in Thailand for which A was measured over a temperature range 23-45 °C. 2) data from an experiment with saplings of rubber trees grown in a growth chamber at 28 °C in France for which A was recorded over a temperature range 9-38 °C. 3) data from an experiment with saplings of rubber tree acclimated to 18 or to 28 °C in a growth chamber in France for which A/C_{i} curves were measured at six different leaf temperatures from 15 °C to 40 °C, to assess changes in photosynthetic capacity with growth temperature. and 4) data from an experiment with 3 clones of 2-year-old rubber trees grown in rubber plantation at Chachoengsao Rubber Research Center.

LITERATURE REVIEW

Photosynthetic carbon metabolism

The photosynthetic processes occur in chloroplasts. Stomata play a central role in transferring CO_2 from the atmosphere into the leaf. Leaf photosynthesis involves two complex series of reactions, the light reactions and the carbon fixation reactions. The energy conversion of photosynthetic apparatus involves light harvesting and light utilisation processes.

The light harvesting is the photochemical process, in which light is absorbed and converted to chemical energy. Light reactions involve the interception of sunlight by reaction centers formed by chlorophylls and other pigments and the transfer of electrons to produce high biochemical energy compounds, adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). This chemical energy is used for carbon reduction in the Calvin cycle in which CO_2 is assimilated.

The light utilisation is the process in which the energy is utilized for carbon assimilation. This process includes the carboxylation and regeneration processes of ribulose-1,5-bisphosphate (RuBP), which are the limiting processes in CO_2 assimilation at saturating light (Farquhar *et al*., 1980). In this process, CO_2 moves through the stomata to the intercellular space and passes the cell wall into the chloroplast. Within the chloroplast CO_2 is bound to RuBP which then undergoes carboxylation catalyzed by the enzyme RuBP carboxylase/oxygenase (Rubisco) for forming triose phosphate and carbohydrate. RuBP is also regenerated into the C_3 pathway (Taiz and Zeiger, 2002).

Biochemical model for leaf photosynthesis

Many models try to describe and predict leaf photosynthesis response to environmental factors, such as light, CO_2 concentration, temperature and air humidity (von Caemmerer, 2000). Among them, the Farquhar model (Farquhar *et al.*, 1980) has had the strongest impact and is widely validated. The Farquhar's model is a simple mechanistic model for studying the temperature response of photosynthesis. This model is based on the kinetics of Rubisco and describes the main reactions in the biochemistry of photosynthesis. The model calculates the photosynthetic rate as the minimum of two limitations, the maximum rate of Rubisco-catalysed carboxylation (Rubisco-limited) or the regeneration of RuBP controlled by electron transport rate (RuBP-limited). The model has two parameters, the maximum rate of electron transport or RuBP regeneration ($J_{\max}$) and the maximum rate Rubisco activity (V_{cmax}) (Farquhar *et al.*, 1980). The estimated parameters $J_{\max}$ and V_{cmax} may be obtained in several methods, e.g. gas exchange and chlorophyll fluorescence (Medlyn *et al.*, 2002a). In gas exchange method, values of $J_{\max}$ and V_{cmax} are obtained from full A/C_{i} data set of the photosynthetic response under high light intensity (A) and intercellular CO_2 (C_{i}).

The parameters of this photosynthetic model vary widely among species (Wullschleger 1993; Dreyer *et al.*, 2001; Leuning, 2002; Medlyn *et al.*, 2002*a*) and within species according to growth conditions (Berry and Björkman, 1980; Ferrar *et al.*, 1989; Bunce, 2000). They are influenced (i) by leaf structure and chemistry, particularly nitrogen content (Harley *et al.*, 1992; Niinemets and Tenhunen 1997; Le Roux *et al.*, 1999; Niinemets *et al.*, 1999b) and (ii) by leaf temperature (Leuning, 1997; Walcroft and Kelliher 1997; Niinemets *et al.*, 1999a; Dreyer *et al.*, 2001; Medlyn *et al.*, 2002a, 2002b).

Temperature response and acclimation of photosynthesis

In nature, plants are exposed to variable environmental conditions. Effects of increasing temperature on plant growth have been extensively studied. Temperature is an important factor affecting all biochemical processes occurring inside a leaf like photosynthesis which is an enzymatic process. The response of photosynthesis to temperature differs significantly with genotype and other environmental conditions (Medlyn *et al.*, 2002a). Immediate response of photosynthetic rate to current conditions of temperature could occur within seconds to minutes. The light-saturated rate of net CO_2 assimilation rate (A_{max}) is reduced at the low and high temperature and has high rate at an optimum temperature (Berry and Björkman 1980, Sage and Kubien, 2007). This optimal temperature depends on species. Increasing temperature caused increasing light-saturated photosynthetic rate, light compensation point, quantum yield and respiration rate (Lewis *et al.*, 1999). This is a short-term response (Thornley, 1998). In general, photosynthesis in tropical species can function without irreversible injury between 15-45 °C (Sage and Kubien, 2007).

In addition to short term effects, growth temperature can induce acclimation of photosynthesis, e.g. change the photosynthetic capacity and the temperature response of photosynthesis (Berry and Björkman, 1980; Bunce, 2000; Medlyn *et al.*, 2002*a*; Bernacchi *et al.*, 2003; Onoda *et al.*, 2005a, 2005b; Yamori *et al.*, 2005; Atkin *et al.*, 2006b; Kattge and Knorr, 2007; Sage and Kubien, 2007; Warren, 2008). Plants are thought to acclimate to the growth temperature to achieve efficient photosynthesis at the new temperature (Berry and Björkman, 1980; Sage and Kubien, 2007). The ability of acclimation of photosynthesis to growth temperature differs among species (Atkin *et al.*, 2006*b*). Most plants grown at lower temperature have lower optimum temperature for net CO_2 assimilation rate (Slatyer, 1977*a*, 1977*b*; Berry and Björkman, 1980; Hikosaka *et al.*, 2006). Some species show phenotypic plasticity which is the capacity to exhibit contrasting phenotypes, i.e. leaf structure and morphology in response to growth at different temperature (Atkin *et al.*, 2006a, and references therein). A component of biomass allocation such as leaf mass per area (LMA) may also be affected by temperature. Plants exposed to low temperature displayed lower LMA than plants grown at warm temperature (Atkin *et al.*, 2006a). Leaf thickness can affect the photosynthetic capacity per unit leaf volume or the quantity of photosynthetic tissue per unit leaf area (Pearcy and Sims 1994, Sims *et al.*, 1998).

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Increasing temperature can cause changes in biochemical mechanism of photosynthesis like rubisco capacity and electron transport capacity (Sage and Kubien, 2007). There is evidence that parameters of the photosynthetic model, V_{cmax}

and J_{max} acclimate to plant growth temperature (Hikosaka *et al.*, 1999; Medlyn *et al.*, 2002b; Bernacchi *et al.*, 2003; Onoda *et al.*, 2005). If temperature responses of the related partial reactions are different, the temperature response of photosynthetic rate will be delimited by the lower one of these temperature responses and the optimal temperature will shift (Yamori *et al.*, 2005). The shift in optimum temperature for photosynthesis may result from change in temperature response of V_{cmax} and J_{max} (Hikosaka *et al.*, 1999; Bunce, 2000; Lloyd and Farquhar, 2008) and change in balance between RuBP carboxylation and regeneration as described by the ratio of J_{max} to V_{cmax} (Farquhar and von Caemmerer, 1982). CO_2 response curves at different temperatures will give the temperature response of J_{max} and V_{cmax}. In most of the studies, temperature response of these parameters was obtained by applying temperature control to leaves during the gas exchange measurements (Medlyn *et al.*, 2002b). For instance, responses of photosynthesis to intercellular CO_2 concentration have been measured at different temperature by Bernacchi *et al.* (2003) in *Pinus radiata*. When temperature increased from 8 to 30 °C, V_{cmax} and J_{max} increased 4-fold and 3-fold, respectively. Temperature optimum for V_{cmax} was higher than J_{max} (Walcroft *et al.*, 1997).

A balance between RuBP carboxylation and regeneration involves nitrogen supply. This implies that leaves balance investment in *N* between the ability for electron transport (A_j) and carboxylation (A_c) which potentially limits photosynthesis. If rates of electron transport and carboxylation are co-limiting, the supply of RuBP by electron transport exactly equals its demand by Rubisco such that the production of unusable RuBP is minimized. Resource allocation that balances limitation by electron transport and carboxylation also minimizes photoinhibition or damage due to the harvesting of excess unusable photons at saturating light levels. Leaves or canopies will adjust to the mean growth conditions so that no one factor of physiological (i.e. A_j, A_c) or environmental (light, temperature, water, nitrogen, CO_2) is more limiting than any another.

The response of the electron transport capacity (J_{max}) to temperature is complex. It is still unclear what determines the capacity of RuBP regeneration. RuBP regeneration rate is generally determined by the electron transport rate in the thylakoid membranes. RuBP regeneration can also be limited by Calvin cycle enzymes. The increase in the whole chain electron transport rate with increasing temperature has been correlated with temperature stimulation of energy flow through photosystem II (PSII), and electron flow from the quinones to photosystem I (PSI). However, the mechanism involves the decline in the electron transport rate above the thermal optimum remains not completely certain (June *et al.*, 2004). Yamasaki *et al.* (2002) demonstrated that the capacity for electron transfer from plastoquinone to P700 declines above the thermal optimum. This shows that electron flow between the photosystems can cause the decline in the electron transport rate. The temperature response of RuBP carboxylation is more stable because kinetic parameters of Rubisco are relatively conserved (von Caemmerer, 2000). If temperature increases, carboxylation capacity changes because of thermal effects on Rubisco, oxygenase activity increases which reflects reductions in both the CO_2/O_2 ratio in solution and the relative specificity of Rubisco for CO_2 (von Caemmerer, 2000).

Variation in photosynthetic capacity in response to a change in growth conditions such as temperature (Berry and Björkman, 1982) can result from shifts in leaf biochemistry and anatomy. These responses may involve changes photosynthetic enzyme concentration, as the maximum rate of RuBP carboxylation (V_{cmax}) increases with increasing rubisco content (Caemmerer and Farquhar, 1981). Decline of optimal temperature at lower temperature can result from reduced enzyme activity and nitrogen partitioning between chlorophyll and Rubisco (Hikosaka, 1997).

The response of V_{cmax} and J_{max} to increasing temperature can be modelled by an Arrhenius function. These functions are based on values of V_{cmax} and J_{max} at a reference temperature of 25 °C ($V_{\mathrm{cmax\,25}}$ and $J_{\mathrm{max\,25}}$). Both parameters varies among species and also within species with variation of nutrient availability, season, leaf age and leaf position within the canopy (Medlyn *et al.*, 1999; Wilson *et al.*, 2000; Misson *et al.*, 2006). Hikosaka *et al.* (2007) showed that the factors of intercellular CO_2 concentration, activation energy of the maximum rate of RuBP (ribulose-1,5-bisphosphate) carboxylation (V_{cmax}), activation energy of the rate of RuBP regeneration ($J_{\max}$), and the ratio of $J_{\max}$ to V_{cmax} may cause the shift of optimal temperature.

Temperature for photosynthesis in rubber

The optimum temperature for photosynthesis in rubber was between 27 and 33 °C (Rao *et al.*, 1998, and references therein). The temperature associated with optimum latex yields of rubber was between 22.8 and 30.4 °C (Rao *et al.*, 1998). Alam *et al.* (2005) showed that rubber trees can adapt to two different agro-climatic conditions. Rubber grown under colder climates (minimum/maximum 8.4/ 23.9 °C in December) had lower net CO_2 assimilation rates and carboxylation efficiency than plants grown under warmer climates (minimum/maximum 20.7/31.9 °C in December). Temperature below 18 °C can be stressful and affects the growth of rubber trees (Alam and Jacob, 2002, and references therein).

Effect of leaf age on photosynthesis

Leaf photosynthesis can be influenced by many plant factors such as leaf age, leaf position, and sink effects, as well as environmental factors such as light, temperature, nutrition, and water availability (Xie and Luo, 2003, and references therein).

Photosynthesis of rubber

There was a difference of maximum net photosynthetic rate ($A_{\max}$) in different rubber clones under tropical field conditions (Ceulemans *et al.*, 1984; Samsuddin and Impens, 1978; Dey *et al.* (1995). The range of net photosynthetic rate in RRIM 600 was 10.2-13.8 µmol m^{-2} s^{-1}. There were the positive correlations between photosynthetic rate, girth and yield in plants after fifth year of tapping (Samsuddin *et al.*, 1987). Photosynthetic rate depends not only on environmental variables, but also on leaf age. Decrease in photosynthetic rate of aging leaves was also associated with decrease in stomatal conductance (Samsuddin and Impens, 1979a; Nataraja and Jacob,1999).

Samsuddin and Impens (1979b) characterized leaf age by leaf blade class (LBC) concept which is based on the growth habit of rubber leaves. For example, a leaf characterized by LBC 9 has the age of about 35 days. Samsuddin and Impens (1979c) showed that photosynthetic rate of *Hevea* remained constant after reached the maximum photosynthetic rate at leaf blade class (LBC) around 6. The rates of net photosynthesis of leaf age between LBC 2 to LBC 6 increased from 1.14 to 5.68 μmol m^{-2} s^{-1}; and LBC 6 to LBC 9 + 40 days, A_{max} decreased from 5.68 to 3.41 μmol m^{-2} s^{-1}. Photosynthesis increases, reaches the maximum rate and falls slowly to what seems to be a constant rate. Decrease in stomatal conductance was also related with the decline in photosynthetic rate of aging leaves.

Nugawela *et al.* (1995c) showed that rubber clones differ in their abilities to maintain net CO_2 assimilation rate under constant saturating light. Nugawela *et al.* (1995a, b) found a diurnal variation of net CO_2 assimilation rate and the internal leaf CO_2 concentration to ambient CO_2 concentration ratio (C_i/C_a). This variation was correlated with stomatal conductance. Plants grown under full light had higher net CO_2 assimilation rate than plants grown under 40% and 25% of incident light. Sun and shade leaves contributed to different dry matter production. Gunasekara *et al.* (2007) found that light-saturated maximum photosynthesis of tapped trees was higher than that of untapped trees. Tapping can increase leaf and canopy photosynthesis as the demand for assimilates from latex-producing tissue increases when latex removes through tapping, thus increased sink demand stimulates assimilate synthesis through photosynthesis.

With concern of elevated CO_2 concentration in the atmosphere and temperature which results in global climate change, there were the studies about the effect of elevated CO_2 concentration on photosynthesis in *Hevea brasiliensis*. Devakumar *et al.* (1998) found that net photosynthetic rate increased with increase in CO_2 concentration. Alam and Jacob (2002) and Alam *et al.* (2005) showed that photosynthesis in rubber can acclimate to different agro-climatic conditions. Low temperature decreased net photosynthetic rate.

Many studies have reported the value of net photosynthetic rate in rubber. However, the value of photosynthetic capacity estimated by using Farquhar's model and whether photosynthesis in rubber can acclimate to temperature which is described by photosynthetic parameters has not been clearly reported. Thus the present study is going to assess the photosynthetic capacity, temperature response and acclimation of photosynthesis in rubber.

MATERIALS AND METHODS

Part I Temperature response and acclimation of photosynthesis of potted-rubber saplings

Materials

Rubber (*Hevea brasiliensis* Muell. Arg.) clone PB 260 and RRIM 600 were selected for this study. These clones display high latex yields and are recommended for commercial plantation by the Rubber Research Institute of Thailand (RRIT, 2003). Gas exchange was measured on intact, fully expanded and mature leaves with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA).

Methods

Gas exchange measurement

Gas exchange measurement was measured on intact, fully expanded and mature leaves with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA). Before making the measurements, CO_2 and H_2O zero was set each day using new sodalime and drierite. Photosynthetic photon flux density (PPFD) was supplied with a red-blue LED light source (LI-6400-02B) and was set above the photosynthetic light saturation point. The CO_2 concentration of the reference air entering the leaf chamber was set at each concentration using the CO_2 mixer. The data were logged when photosynthesis was stable.

Experiment 1. Temperature response of net CO_2 assimilation of rubber sapling grown in nursery in Thailand

This study was carried out during 2005-2006. Budded scions of rubber clone RRIM 600 were planted in 50 litre pots filled with Pakchong soil series composed of sand (24.5 %), silt (6.3 %), clay (69.2 %) and organic matter (2.7 %). All plants were grown in a nursery under natural conditions at Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand (latitude:13.85 N, longitude:100.75 E). Ambient temperature was recorded with WatchDog model 900ET Weather Station (Spectrum technologies, Inc). The ambient temperature varied between 27-38 °C in a day during the study period. All plants were well-irrigated daily. Four three-month-old rubber plants were used in the experiment.

Temperature response of net CO_2 assimilation (A) was measured between 23 and 45 °C in the nursery. To obtain the lowest temperatures, the ambient air temperature was decreased by cooling with custom air-temperature control system constructed at Kasetsart University (Figure 1). The cooling system consisted of an air-conditioning system and an air blower installed inside a closed-container. The closed-container was built with autoclaved aerated concrete, Q-CON which offered a thermal insulation. The cool air was pulled out from the container with the air blower and passed through a 2-inch-diameter PVC ducting hose covered with thermal insulation to cool the part of sensor head of LI-6400. The sensor head, IRGAs and leaf chamber were covered with transparent plastic bag in which cool air was blown. The cooling air entered also the inlet of console of LI-6400 through a 6-mm-diameter PVC plastic pipe. The temperature of the air regulation system could be set between 17 and 30 °C. The low leaf temperature during gas exchange measurement was controlled with the air regulation system. Additionally, leaf temperature during the photosynthesis measurement was controlled through the Peltier element of LI-6400. Leaf photosynthesis was measured while leaf temperature was increased gradually at 1-2 °C intervals between 23 and 45 °C or between the minimum and maximum of temperature that could be obtained during the measurements. Photosynthesis measurements were logged after 15-20 minutes stabilization at each temperature step. Measurements were made on 4 plants (one leaf of each plant) at light saturation of 1,200 μmol m^{-2} s^{-1} PPFD and ambient CO_2 concentration of 380 μmol mol^{-1}.

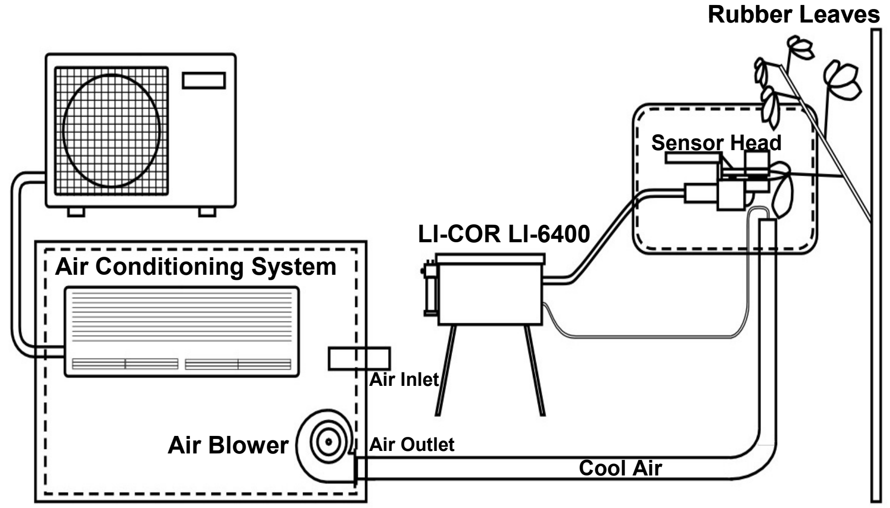


Figure 1 Schematic diagram of an air-temperature control system constructed at Kasetsart University, Thailand. Ambient air was cooled before entering the LI-6400 system during gas exchange measurement.

Experiment 2. Temperature response of *A* of rubber grown in growth chamber

This study was carried out during 2006. One sapling of rubber clone PB 260 was obtained from Michelin Company in Clermont-Ferrand, France. Plant was grown in a large containers (33 litre) filled with a 1:2 (v/v) mixture of peat and clay soil (fertile soil and manure). The potted-plant was acclimated in a growth chamber at PIAF INRA, Clermont-Ferrand, France. The growth chamber was set at 28 °C, 12h photoperiod with 250 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at leaf height and 80 % relative humidity. The plant was well-irrigated every day. Plant was fertilized two-three times a week with 1.5 litre Plant-Prod 20-20-20 water soluble fertilizer (0.5 g of Plant-Prod/ litre of water) (Plant Products Co.Ltd., Ontario, Canada).

Plant grown at 28°C (day and night) during 2 months was then transferred to another growth chamber in which ambient temperature was controlled for the measurements. Temperature response of photosynthesis was obtained by controlling the ambient temperature in the growth chamber and the leaf temperature in the Licor LI-6400 leaf chamber at the target temperature. Photosynthesis measurements were made on 6 leaves of one plant at 1-2 °C intervals between 9 and 38 °C. The growth chamber was set at 10 ±1, 20 ±1 and 32 ±1 °C for leaf temperature ranges of 9-16 °C, 17-25 °C and 26-38 °C respectively. At each temperature step, photosynthesis measurements were logged after 5-10 minutes stabilization. The leaf temperature was increased gradually at 1-2 °C intervals between 9 and 38 °C. Photosynthesis was measured at saturating light (1,200 μmol m^{-2} s^{-1} PPFD) and ambient CO_2 concentration of 350 μmol mol^{-1}.

Experiment 3. Temperature response and acclimation of *A*, V_{cmax} and J_{max} of rubber acclimated to different temperatures

The experiment was performed during 2007 at PIAF INRA, Clermont-Ferrand, France. Four potted-plant of three-month-old rubber clones PB 260 and RRIM 600 (two plants of each clone) obtained from Michelin Company were grown in large containers (33 litre) filled with a 1:2 (v/v) mixture of peat and clay soil (fertile soil and manure) and continuously drip-irrigated in a controlled greenhouse at 28 °C/20 °C (day/night) under a 12 h photoperiod (200 μmol photons m^{-2} s^{-1}) at a relative humidity of 80 % (day/night) at Blaise Pascal University, Clermont-Ferrand, France 2 months before the experiment.

Temperature acclimation treatment

Individuals of the two clones were transferred from the greenhouse at 28 °C to two growth chambers for acclimation. One pot of PB 260 and RRIM 600 was transferred to 18 ˚C of one growth chamber and other pot of the same clone was transferred to another growth chamber at 28 °C. The growth temperature of 28 °C was chosen because rubber could be grown well at this temperature. The growth temperature of 18 °C was chosen to be low temperature for acclimation because rubber which is a tropical tree was expected to be grown without damage. The difference between 18 and 28 °C was large enough to elicit a temperature acclimation effect. Both growth chambers were set at 18 and 28 °C respectively day and night, 12/12h photoperiod with 200-250 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at leaf height, and 80 % relative humidity. The plants were well-irrigated every day and fertilized two-three times a week with 1.5 litre Plant-Prod 20-20-20 water soluble fertilizer (0.5 g of Plant-Prod/ litre of water) (Plant Products Co.Ltd., Ontario, Canada).

Plants were acclimated to growth temperature for at least three weeks before starting measurements. Gas exchange was measured on intact leaves that were already fully expanded before temperature acclimation treatment.

CO_2 response curve

CO_2 response curves (A/C_i curves) measurement started from ambient CO_2 concentration at CO_2 360 µmol mol^{-1}. The measurement was recorded when net CO_2 assimilation rate (A) and stomatal conductance (g_s) stabilized by checking the strip chart of photosynthesis and stomatal conductance as a function of time. The first measurement began after about 20-30 minutes until A and g_s were stable, 3 measurements were logged at 15-20 seconds interval. Then CO_2 was changed immediately to the next step and the measurement proceeded again. CO_2 was first decreased step by step to 300, 250, 200, 150, 100 and 50 µmol mol^{-1}. Then CO_2 was set back to 360 µmol mol^{-1} and increased step by step to 450, 600, 800, 1000, 1200, 1400 and 1600 µmol mol^{-1} or more higher values until g_s decreased below 0.040 mol m^{-2} s^{-1}, at which value data were not reliable. The stomatal conductance of these plants decreased quickly at high CO_2. If the CO_2 response curve didn't have enough points (5-6 points) to get the RuBP regeneration limited fraction of the curve (A_j part) because of too low g_s at high CO_2, CO_2 was changed to a low value (50 - 250 µmol mol^{-1}) and waited until g_s increased more than 0.040 mol.m^{-2} s^{-1} and stabilized and the measurement at high CO_2 was proceeded again to get the points of A_j part. One curve was taken from one and a half to three hours to be achieved.

Temperature response and acclimation of A, V_{cmax} and J_{max}

The measurements of A/C_i curves at each temperature were carried out by transferring the plant from the acclimation growth chamber to a third growth chamber for measurement. In the morning, the individual and LI-6400 system were moved to the measurement chamber and kept for at least one hour for equilibration at new temperature before gas exchange measurement were performed. The plant was then moved back to the acclimation temperature after the end of measurement sequence each day.

Temperature response was obtained by applying temperature control to leaves for the duration of the gas exchange measurements. The A/C_i curve of each leaf of individuals was measured at leaf temperature of 15, 22, 28, 32, 36 and 40 °C respectively, except PB 260 acclimated to 18 ˚C which was measured at 15, 22, 28 and 32 °C of leaf temperature because of too low stomatal conductances at higher temperatures. The leaf temperature for all measurements were controlled within $\pm$ 0.5 °C of the target temperature. One A/C_i curve was performed at each leaf temperature. The same leaf was measured to obtain A/C_i curve at all leaf temperatures. Five leaves of PB 260 acclimated to 28 °C and four leaves of PB 260 acclimated to 18 °C, RRIM 600 acclimated to 18 and 28 °C each individual were measured at each temperature. Photosynthetic Photon flux density (PPFD) was controlled at 1,200 μmol m^{-2} s^{-1} which was above photosynthetic light saturation point for all A/C_i curves, except the curves at 22, 28 and 32 °C of PB 260 acclimated to 18 °C where PPFD was set at 800 μmol m^{-2} s^{-1}. The light saturation point of this plant was lower than other plants.

All leaves were collected after the last gas exchange measurement. Leaf greenness was measured with SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan). At the end of the measurements, the leaves of plants acclimated to 18 °C turned to be less green. Leaf characteristics such as SPAD had not been measured at the beginning of the experiment. Leaf area was measured with leaf area meter, Li-3100A (LI-COR Inc., Lincoln, NE, USA). Leaves were dried at 70 °C for

48 hours to obtain dry weight. The nitrogen content was analyzed with an elemental microanalyser (Carlo Erba, model EA 1108, Milan, Italy).

Data analysis

Temperature response of net CO_2 assimilation rate

The response curves of net CO_2 assimilation rate at ambient CO_2 concentration to temperature were fitted by non linear least squares regression of June 's empirical model (June *et al.*, 2004):

$$P(T) = P\left(T_{opt}\right) e^{-\left(\frac{T_{opt} - T}{\Omega}\right)^2} \qquad \textbf{(1)}$$

where T_o = optimal temperature (°C), T = leaf temperature (°C), Ω = shape parameter, P(T) and P(T_o) values of the parameter of interest at leaf temperature and optimal temperature respectively.

CO_2 response curves fit

CO_2 response curves were fitted according to Farquhar *et al.* model (Farquhar *et al.*, 1980; Farquhar and von Caemmerer, 1982; Harley and Tenhunen, 1991) where net assimilation A_n is limited either by activity of Rubisco at saturating RuBP (A_c) or by RuBP concentration (A_j) :

$$A_n = \min(A_c, A_j) \qquad \textbf{(2)}$$

and:

$$A_c = V_{c\max} \frac{C_c - \Gamma^*}{C_c + K_c\left(1 + \frac{O}{K_o}\right)} - R_d \qquad \textbf{(3)}$$

where V_{cmax} (μmol.m^{-2} s^{-1}) is the maximum rate of carboxylation, C_c and O (μbar) the CO_2 and O_2 partial pressures at the carboxylation sites, R_d (μmol m^{-2} s^{-1}) the mitochondrial respiration, K_c and K_o (μbar) the Michaelis-Menten constants for carboxylation and oxygenation respectively, and Γ* (μbar) the CO_2 compensation point in absence of Rd.

$$A_j = J\frac{C_c - \Gamma^*}{4(C_c + 2\Gamma^*)} - R_d \qquad \textbf{(4)}$$

where J (μmol m^{-2} s^{-1}) is the potential electron transport rate.

The dependency of *J* to light was modelled following the empirical relation from Tenhunen *et al.*, 1971):

$$J = \frac{\alpha Q}{\sqrt{1 + \frac{\alpha^2 Q^2}{J_{\max}{}^2}}} \qquad \textbf{(5)}$$

where α is the quantum utilization efficiency, Q (μmol m^{-2} s^{-1}) the incident photon flux density and $J_{\max}$ (μmol m^{-2} s^{-1}) the light saturated potential electron flux.

The temperature dependencies of K_c, K_o and Γ* were modelled according to the Arrhenius equation :

$$P(T) = P\left(T_{ref}\right) e^{\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \qquad \textbf{(6)}$$

where P is the parameter of interest (K_c, K_o or Γ*), T_{ref} the reference temperature (298.15 K), P(T_{ref}) the parameter of interest at reference temperature, E_a the activation energy (J.mol^{-1}), R the gas constant (8.314 J.K^{-1} mol^{-1}) and T the leaf temperature (K).

Estimation of apparent V_{cmax} and J_{max}

The CO_2 response curves were fitted by non linear least squares regression according to Dreyer *et al*., 2001, assuming infinite internal conductance $g_{i,}$ or C_c, CO_2 partial pressure at the carboxylation sites equals C_i, CO_2 partial pressure in substomatal cavities. The parameter set from Bernacchi *et al*. (2001) (Table 1), suitable for A/C_i curve fitting with infinite g_i, was used to estimate V_{cmax} and J_{max} from A/C_i curves.

The fitting procedure using R software yielded therefore estimates of apparent V_{cmax} and J_{max} (Ethier & Livingston, 2004). An attempt to fit A/C_i curve while assuming and then estimating finite g_i as described in Ethier and Livingston (2004) did not turn to be reliable and was then given up.

Temperature response of photosynthetic model parameters (V_{cmax} and J_{max})

The response curves of photosynthetic model parameters (V_{cmax}, J_{max} and the ratio J_{max}/V_{cmax}) were fitted with the Arrhenius function (equation 6) by weighted non linear least squares fit. Data points (*i.e.* estimates of apparent V_{cmax} or J_{max} at each measurement temperature) were weighted by the reciprocal of their estimation variances obtained from the fit of CO_2 response curves.

In order to test clone and acclimation temperature effect, the estimates from all plants and leaves were pooled in one single fit, using dummy variables for each plant and each leaf, following the procedure described in Dreyer *et al*., 2001, so as to get an estimate of the parameter (V_{cmax} or J_{max}) at 25 °C for each leaf and an estimate of activation energy for each clone x acclimation temperature combination. It is therefore assumed that leaves from the same clone under the same acclimation temperature share identical activation energies. Then an analysis of contrasts was performed for testing specific hypotheses.

A similar procedure using dummy variables and pooled data in experiment 2 was used for fitting June *et al*. model (equation 1), here assuming same optimal temperature and shape parameter for all leaves.

Primary model parameters K_c, K_o, τ and α (apparent quantum yield) are listed with their temperature dependencies in Table 1. Primary data were taken from Bernacchi *et al*. (2001).

Table 1 Data set of primary parameters and their temperature response from Bernacchi *et al*. (2001) used to estimate V_{cmax} and J_{max} from A/C_i curves.

Parameter	units	Values used here
α	mol (electrons) mol^{-1} (photons)	0.24
K_c (25 °C)	microbar	404.9
E_a (Kc)	J mol^{-1}	79,430
K_o (25 °C)	microbar	278,400
E_a (Ko)	J mol^{-1}	36,380
τ (25 °C)	–	42.75
E_a (τ)	J mol^{-1}	37,830
O	microbar	210,000

Part II

Experiment 4 Effect of leaf age and season on photosynthesis of 2-year-old field-grown rubber trees

Plant materials and study site

Field site was located at Chachoengsao Rubber Research Center in eastern Thailand (13° 41’ N, 101°04’ E, 69 m above sea level). Rubber was planted in a field clone trial in June 2004. Spacing was 3 x 7 m. Soil on the site was Kabin Buri series.

Trees were 2-year-old at the beginning of the experiment. Rubber clones PB 260, RRIM 600 and RRIT 251 were chosen for this study (clonal buds grafted on uncontrolled seedling rootstocks). These clones produce high latex yield and are categorized in the first class which is suitable for large scale planting. They are recommended for commercial plantation by the Rubber Research Institute of Thailand (RRIT, 2003). Air temperature and relative humidity were recorded semi-hourly with weather monitoring system (Vaisala Inc., MA, USA).

Photosynthetic measurements were made every two months in October 2006, December 2006 and April 2007, except February 2008 which all leaves dropped as a natural defoliation period of rubber tree. Photosynthetic measurements were made from 3rd to 28th October 2006 (wet and rainy season), from 21st to 29th December 2006 (beginning of dry season) and from 29th March to 5th April 2007 (beginning of rainy season). The measurements were performed every day in each period of study. The tree height was around 4.5- 6 m high and there was no inter-tree shading. A portable scaffolding tower was erected around rubber trees during the measurements.

As rubber shoots grow rhythmically (Thaler and Pages, 1994, and references therein), developing a new growth unit made of a shoot section and a whorl of leaves every about 30-45 days, whorl position is an indicator of both leaf age and leaf position within the crown. Therefore it is an appropriate parameter to classify leaves in the purpose of modelling photosynthesis on a canopy scale. Each whorl is considered a class of leaves. The oldest whorl (bottom whorl) was named "whorl 1" or W1, the next was named "whorl 2" (W2), then "whorl 3, 4 and 5" (W3, W4, W5) until the youngest whorl. In October, every measurement was conducted on 7-8 fully expanded leaves of all whorls of each clone.

All leaves measured in October were marked for the next measurement on the same leaves in December to minimize variability in photosynthesis. The measurements in December were made on 8-12 fully expanded leaves of each clone. In April, there was only one whorl of leaves, as trees lost completely their leaf in February and re-foliated thereafter. Measurements were made on 13-16 fully expanded leaves of each clone.

Photosynthetic measurements

Leaf gas exchange measurement was measured with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA). Light was supplied with 6400-02B LED light source. The CO_2 concentration of the reference air entering the leaf chamber was controlled with a CO_2 mixer. The chamber temperature was be controlled by maintaining the Peltier block temperature.

Maximum net CO_2 assimilation rate (A_{max}) of different leaf age whorls were measured under ambient CO_2 concentration of 360 µmol mol-1 and at saturating ambient irradiances, photosynthetic photon flux density (PPFD) of 1600 µmol m^{-2} s^{-1}.

The response of net photosynthesis (A) to intercellular leaf CO_2 concentration (C_i) was determined at saturating light intensity (1600 µmol m^{-2} s^{-1}). The measurement was started at ambient CO_2 concentration of 360 µmol mol^{-1} and allowed to equilibrate to reach a steady state at least 15 minutes. The measurement was recorded when net CO_2 assimilation rate (A) and stomatal conductance (g_s) stabilized. The CO_2 was decreased stepwise to 250, 200, 150, 100 and 50 µmol mol^{-1} and then increased stepwise from 360 to 600, 800, 1000, 1100, 1200, 1400 and 1600 µmol mol^{-1} to obtain A_c and A_j (RuBP carboxylation and regeneration limited part of A/C_i curve, respectively) parts of each full curve. Leaf temperature was maintained at constant ambient levels during the measurement cycle. A/C_i curve was produced by plotting net CO_2 assimilation against the intercellular leaf CO_2 concentration.

Estimation of photosynthetic parameters

CO_2 response curves were fitted as in pot experiments (see above).

The values of V_{cmax} (maximum carboxylation rate) and J_{max} (maximum electron transport rate) were estimated by fitting the Farquhar's model to the Rubisco limited portion (A_c) and to the RuBP regeneration limited portion (A_j) of the A/C_i curves at low C_i and at saturating C_i level, respectively.

All estimated photosynthetic parameters (V_{cmax} and J_{max}) measured at all temperatures were normalized to the values at a reference temperature of 25 °C.

Normalization of estimated photosynthetic capacity of rubber trees in rubber field

The estimated values of V_{cmax} and J_{max} in 2-year-old rubber trees grown in rubber plantation at CRRC were normalized to the values at a reference temperature of 25 °C by calculating with the following equations:

$$P\left(T_{ref}\right) = P\left(T\right) e^{-\left(\frac{E_a}{R}\right)\left[\frac{1}{T_{ref}} - \frac{1}{T}\right]}$$

where P is the estimated parameter of V_{cmax} and J_{max} from the measurement, T_{ref} the reference temperature (298.15 K, 25 °C), P(T_{ref}) the parameter of interest at reference temperature, E_a the activation energy (J.mol^{-1}) of rubber saplings from the experiment 3 in Part I, over the range 15-40 °C (E_a of V_{cmax} and E_a of J_{max} of RRIM 600 grown at 28 °C were 66163 and 46017 J mol^{-1}, respectively. E_a of V_{cmax} and E_a of J_{max} of PB

260 grown at 28 °C were 70866 and 55206 J mol^{-1}, respectively.), R the gas constant (8.314 $J.K^{-1}.mol^{-1}$) and T the leaf temperature (K).

Leaf mass per area and nitrogen analysis

At the end of the measurement, leaves were collected. Chlorophyll content was assessed with a portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan). Leaf area was measured with a portable leaf area meter, LI-3100A (LI-COR Inc., Lincoln, NE, USA). Leaves were dried at 70 °C for at least 48 hours to assess constant dry mass. Leaf mass per area (LMA) was calculated from measurements of leaf area and dry weight. Leaf sample was ground and nitrogen content was analyzed with a nitrogen determinator model FP-528 (Leco corporation, Michigan, USA).

Data analysis

For each rubber clones, analysis of variance, statistical difference in measured data (SPAD, A_{max},dw, LMA, N_{m}, N_{a} and PNUE) and estimated parameters (V_{cmax}, J_{max}, V_{cmax25} and J_{max25}) of physiological traits across whorl number were assessed using Statistical Analysis System (SAS Institute Inc., USA).Duncan’s mean separation test was performed for significant whorl (age) effect. Standard errors of mean of measurement parameters were also analyzed using SAS.

For each whorl number, effect of season on measured data and estimated parameters of physiological traits were tested using GLM procedure of Statistical Analysis System (SAS, SAS Institute Inc., USA). The means were compared with Duncan’s mean separation.

RESULTS AND DISCUSSION

Results

Part I Photosynthesis in potted-rubber saplings

Experiment 1 Temperature response of net CO_2 assimilation (*A*) of rubber sapling grown in nursery in Thailand

Figure 2 (experiment 1) showed the response of *A* of 4 rubber plants clone 600 grown in a nursery in Thailand to temperature ranged 23-45 °C. Plants experienced growth temperature ranged 27-38 °C in a day. Four plants showed a similar trend of temperature response of *A*. The optimal temperatures of *A* of these plants were in a very narrow range between 28.3 and 30.3 °C (Table 2). The shape parameter (Ω) of 3 plants was between 19.3 – 20.6 °C, except one plant was 12.7 °C.

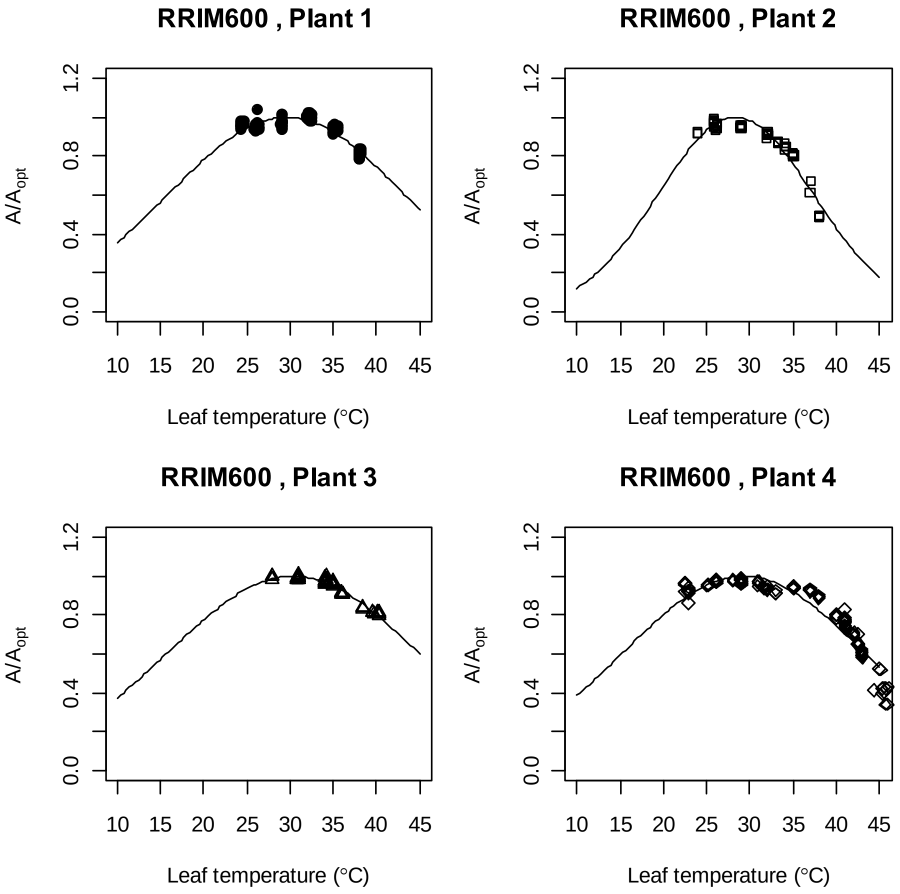


Figure 2 Temperature response of net CO_2 assimilation rate (*A*) relative to values at optimum temperature (A_{opt}) of 4 plants of RRIM 600 grown in a nursery. Leaf temperature ranged from 23 to 45 °C. *A* was measured at an ambient CO_2 mole fraction of 380 µmol mol^{-1}.

Experiment 2 Temperature response of *A* (net CO_2 assimilation rate) of rubber grown in growth chamber

The temperature response of net CO_2 assimilation of 6 leaves of PB 260 (one plant) grown in the growth chamber was similar. The temperature ranged 9-38 °C. The mean optimal temperature of *A* was 27.8 °C. Interestingly, it was very close to growth temperature in growth chamber which was 28 °C. The Ω was 19.2 °C (Figure 3, Table 2).

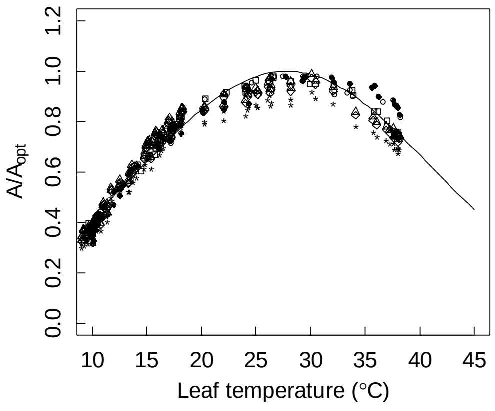


Figure 3 Temperature response of net CO_2 assimilation rate (*A*) relative to values at optimum temperature (A_{opt}) of 6 leaves in PB 260 grown in a growth chamber at 28 °C. Leaf temperature ranged from 9 to 38 °C. *A* was measured at an ambient CO_2 mole fraction of 350 μmol mol^{-1}. Different symbols represent different leaves.

Experiment 3 Temperature response and acclimation of *A* (net CO_2 assimilation rate), V_{cmax} and J_{max} of rubber acclimated to different temperatures

Figure 4 (experiment 3) showed the temperature response of *A* of individuals of PB 260 and RRIM 600 acclimated to 18 and 28 °C. Plant acclimated to different temperatures had significantly different optimal temperature of *A* (Figure 4, Table 2). Plants grown at lower temperature (18 °C) had lower optimal temperature of A than plants grown at higher temperature (28 °C). Clones PB 260 and RRIM 600 acclimated to 28 °C had optimal temperature of *A* at 31.1 and 29.6 °C respectively. Clone PB 260 acclimated to 18 °C had optimal temperature of *A* at 22.3 °C which was close to growth temperature; while RRIM 600 acclimated to 18 °C had optimal temperature of *A* at 27.6 °C. The optimal temperature of *A* of PB 260 acclimated to 18 °C was low and close to growth temperature, but RRIM 600 acclimated to 18 °C have optimal temperature of *A* close to plant acclimated to 28 °C. The clone PB 260 seemed to have more plasticity to acclimation temperature than RRIM 600. The Ω of the individuals of both clones that acclimated to 18 °C was less than individuals that acclimated to 28 °C.

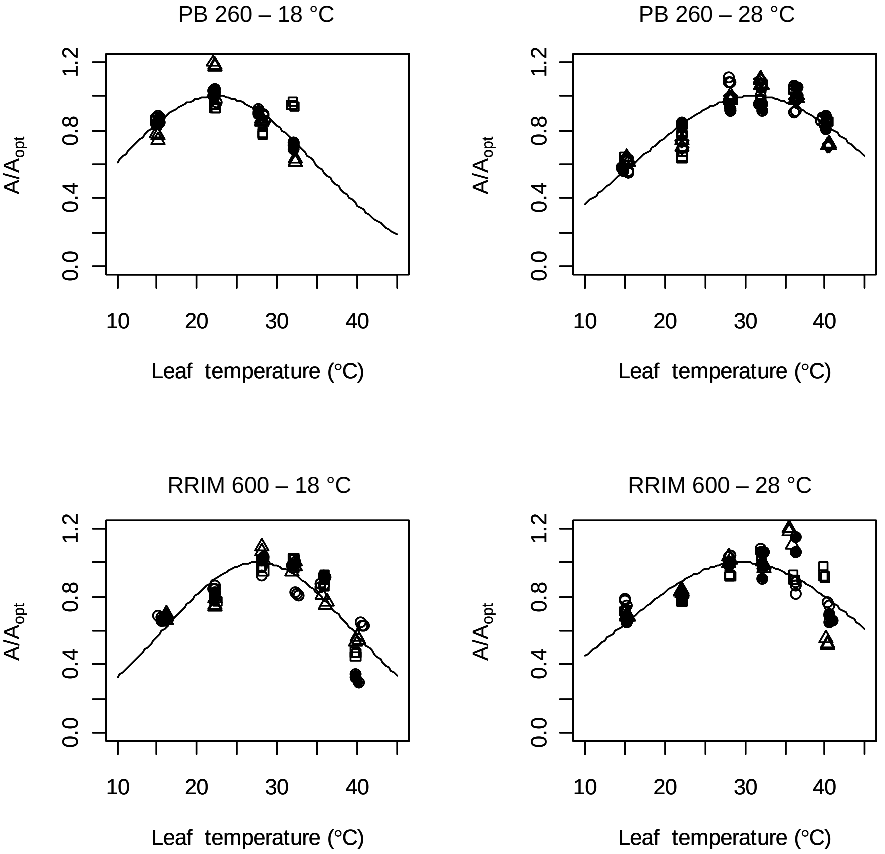


Figure 4 Temperature response of net CO_2 assimilation rate (*A*) relative to values at optimum temperature (A_{opt}) of PB 260 and RRIM 600 grown in a growth chamber at 18 °C and 28 °C. Leaf temperature ranged from 15 to 40 °C. *A* was measured at an ambient CO_2 mole fraction of 360 µmol mol^{-1}. Different symbols represent different leaves.

Table 2 Mean values ± standard error, SE (n = 4-6) of optimum temperature for net CO$_2$ assimilation (T_{opt}) at ambient CO$_2$ concentration of 360 μmol mol^{-1} of rubber saplings grown at different temperatures (T_{growth}), of the shape coefficient of the temperature response (Ω) and of net CO$_2$ assimilation (A) at T_{opt} (A_{opt}). Different superscript letters within the same clone indicate statistically difference values at P<0.001 (except T_{opt} of A of RRIM 600, significant levels at P<0.05) using contrast analysis. Exp1 and 2 are the experiments of temperature response of net CO$_2$ assimilation of rubber saplings grown in a nursery in Thailand and in growth chamber, respectively. Exp3 is the experiment of temperature response of A, V_{cmax} and J_{max} of rubber saplings acclimated to different temperatures.

	Exp1	Exp 2	Exp3			
Sapling	RRIM 600	PB 260	PB 260		RRIM 600	
Growth condition	Nursery, Thailand	Growth chamber	Growth chamber		Growth chamber	
T_{growth} (°C)	27-38	28	18	28	18	28
T_{opt} (°C)	29.4 ± 0.1	27.8 ± 0.1	22.3 ± 0.6^{a}	31.1 ± 0.3^{b}	27.6 ± 0.4^{a}	29.6 ± 0.4^{b}
Ω	18.1 ± 0.3	19.2 ± 0.2	17.6 ± 1.6^{a}	21.1 ± 0.7^{a}	16.7 ± 1.0^{a}	21.9 ± 1.1^{b}
A_{opt}	10.1 ± 0.02	9.0 ± 0.2	5.8 ± 0.2^{a}	10.7 ± 0.1^{b}	4.8 ± 0.1^{a}	7.7 ± 0.1^{b}

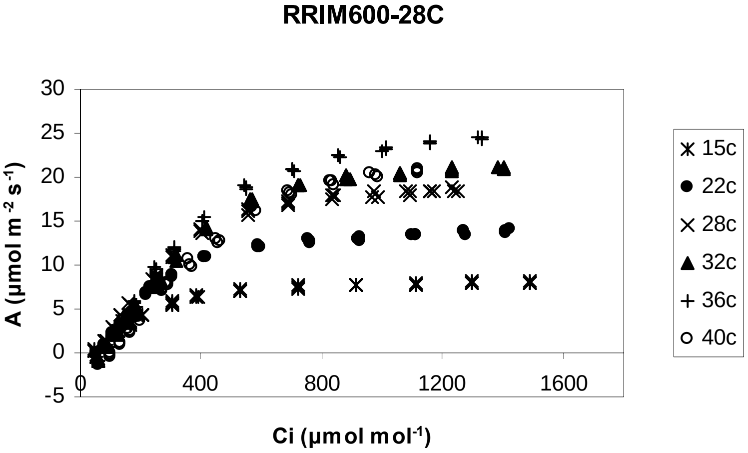


Figure 5 Example of a set of A/C_i curves at six temperatures in a leaf of individual, RRIM 600 grown in a growth chamber at 28 °C. Leaf temperature ranged from 15 to 40 °C. Different symbols represent different leaf temperatures.

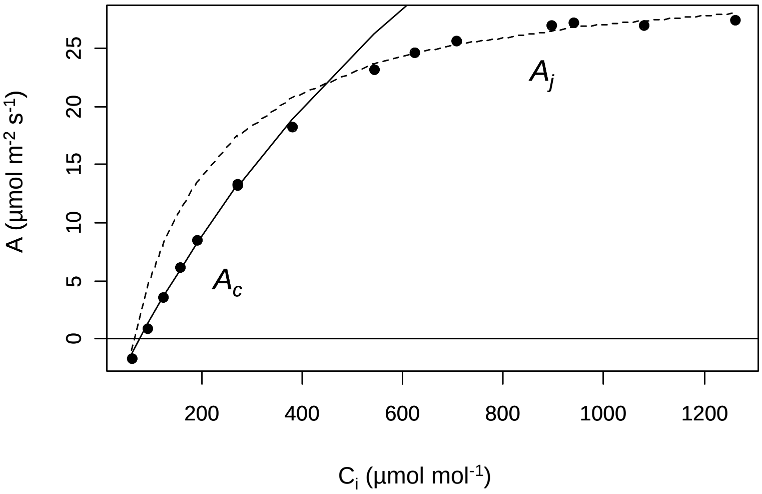


Figure 6 Example of the response functions (Farquhar's model) fitted to data of A/C_i curve; A_c and A_j are the net assimilation rates limited by RuBP regeneration and by carboxylation, respectively.

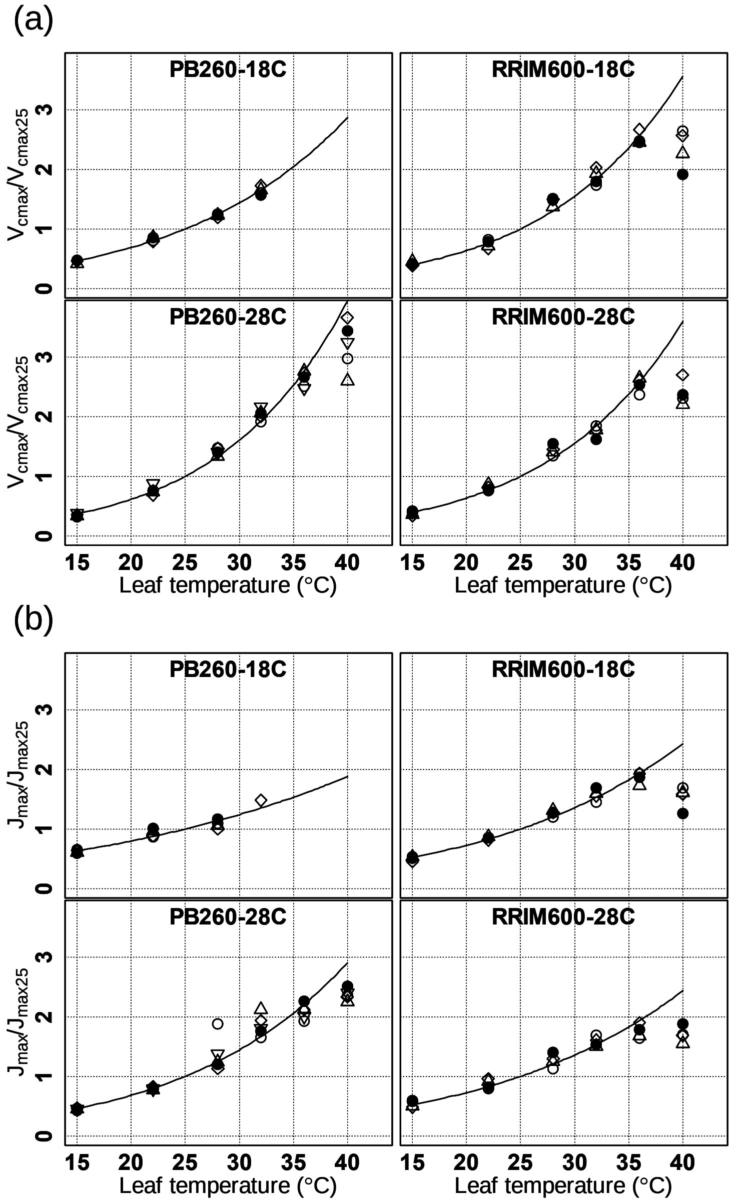


Figure 7 Temperature response of (a) V_{cmax} relative to V_{cmax25} and (b) of J_{max} relative to J_{max25} for rubber saplings acclimated to 18 or to 28 °C. The values of V_{cmax} and J_{max} were estimated by fitting the temperature response functions to A/C_i curves obtained at 6 different temperatures. V_{cmax} and J_{max} were estimated at six different temperatures ranged from 15 to 40 °C and normalized to the mean value at 25 ºC (V_{cmax25} and J_{max25}; n= 4-5). Different symbols represent different leaves.

The V_{cmax} and J_{max} estimates obtained from A/C_i curves at 40 °C (Figure 7) were discarded as an inflexion of their temperature response curves was apparent at this temperature but not enough points were available to accurately estimate the deactivation energy nor the entropy terms needed to account for deactivation at high temperature (Niinemets & Tenhunen, 1997; Dreyer *et al.*, 2001; Medlyn *et al.*, 2002) nor the optimal temperature from empirical June's model. It is therefore assumed that negligible deactivation occurs at temperatures below 36 °C.

A/C_i curves were measured at different temperatures. An example of a data set used to fit the model parameters V_{cmax} (maximum carboxylation rate) and J_{max} (maximum electron transport rate) in a leaf of each individual, PB 260 and RRIM 600 grown in a growth chamber at 18 °C and 28 °C is presented in Figure 5. The example of the fit of the Farquhar’s model to these data is shown in Figure 6.

Rubber clones PB 260 and RRIM 600 acclimated to 18 and 28 °C showed the response of photosynthetic capacity (V_{cmax} and J_{max}) to temperature ranged 15-40 °C by increasing the values with increasing measurement temperatures (Figure 7a, b). The temperature responses above 36 °C of two clones showed two general features.

The mean values of V_{cmax} and J_{max} at 25 °C normalized by adjusting an Arrhenius functions on the temperature response of V_{cmax} and J_{max} in both clones differed between plants acclimated to different temperatures (Figure 7a, b). Plants grown at higher temperature had higher value of both parameters than plants grown at lower temperature. The apparent V_{cmax} (25 °C) of PB 260 acclimated to 18 and 28 °C were 30.7 and 49.0 µmol m^{-2} s^{-1}, respectively and of RRIM 600 were 21.5 µmol m^{-2} s^{-1} to.38.7 µmol m^{-2} s^{-1}, respectively. The apparent J_{max} (25 °C) of PB 260 acclimated to 18 and 28 °C were 59.8 and 89.3 µmol m^{-2} s^{-1}, respectively and of RRIM 600 were 41.5 µmol m^{-2} s^{-1} to 65.5 µmol m^{-2} s^{-1}, respectively (Figure 8).

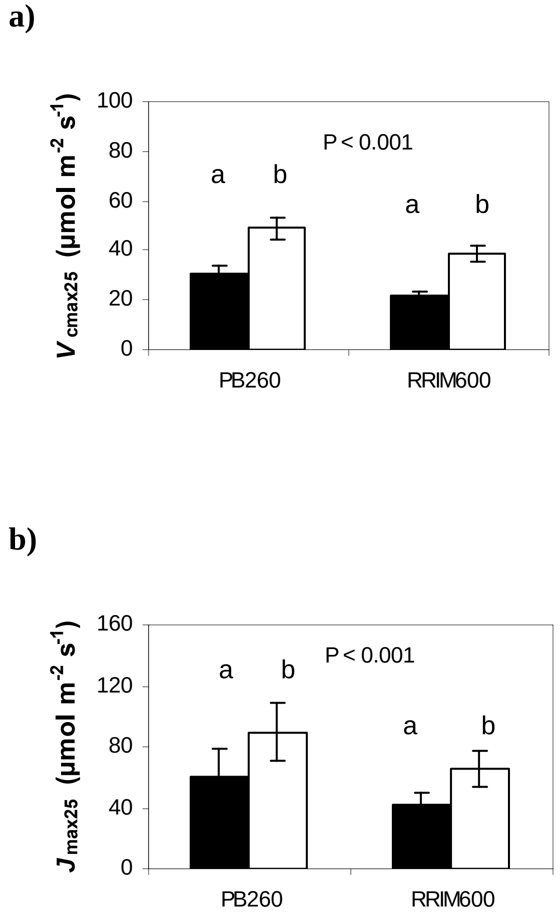


Figure 8 (a) Mean values of apparent V_{cmax25} in rubber saplings acclimated to 18 °C (closed columns) and 28 °C (open columns). (b) Mean values of apparent J_{max25} in rubber saplings acclimated to 18 °C (closed columns) and 28 °C (open columns). The values of V_{cmax25} and J_{max25} were estimated by adjustment of Arrhenius functions to the normalized temperature response of V_{cmax} and J_{max}. Bars represent $\pm$ SE (n = 4-5). Different letters above the column indicate statistical difference between different growth temperatures of the same clone. Significant level: $P<0.001$.

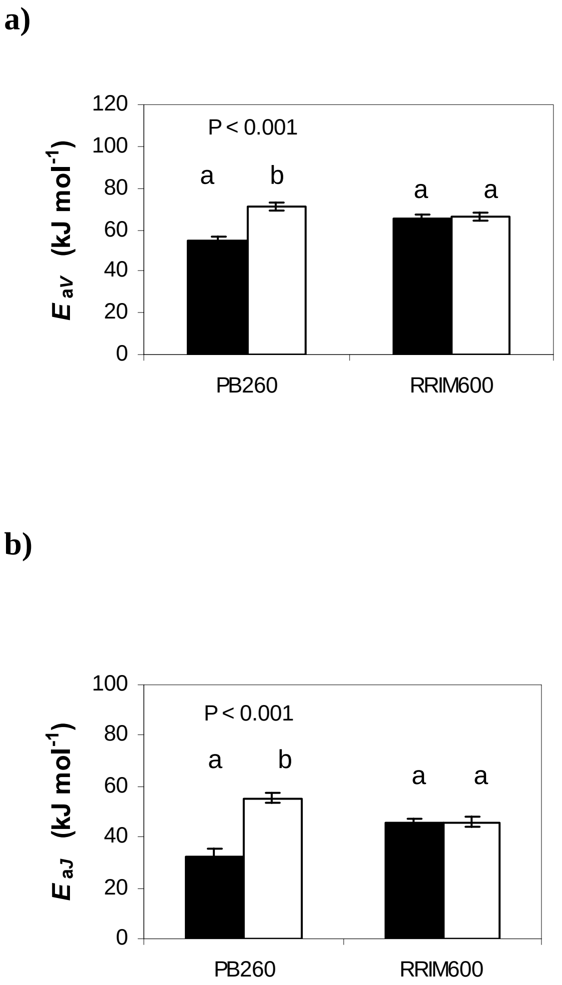


Figure 9 (a) Mean values of activation energy of V_{cmax} (E_{aV}) in rubber saplings acclimated to 18 °C (closed columns) and 28 °C (open columns). (b) Mean values of activation energy of J_{max} (E_{aJ}) in rubber saplings acclimated to 18 °C (closed columns) and 28 °C (open columns). The values of E_{aV} and E_{aJ} were estimated by adjustment of Arrhenius functions to the normalized temperature response of V_{cmax} and J_{max}. Bars represent $\pm$ SE (n = 4-5). Different letters above the column indicate statistical difference between different growth temperatures of the same clone. Significant level: $P<0.001$.

The values of activation energies of V_{cmax} and J_{max} obtained by adjusting an Arrhenius function on the temperature response of V_{cmax} and J_{max} of PB 260 acclimated to 28 °C were significantly higher than for the plant acclimated to 18 °C. Whereas these parameters for RRIM 600 acclimated to 18 and 28 °C did not differed significantly (Figure 9a, b).

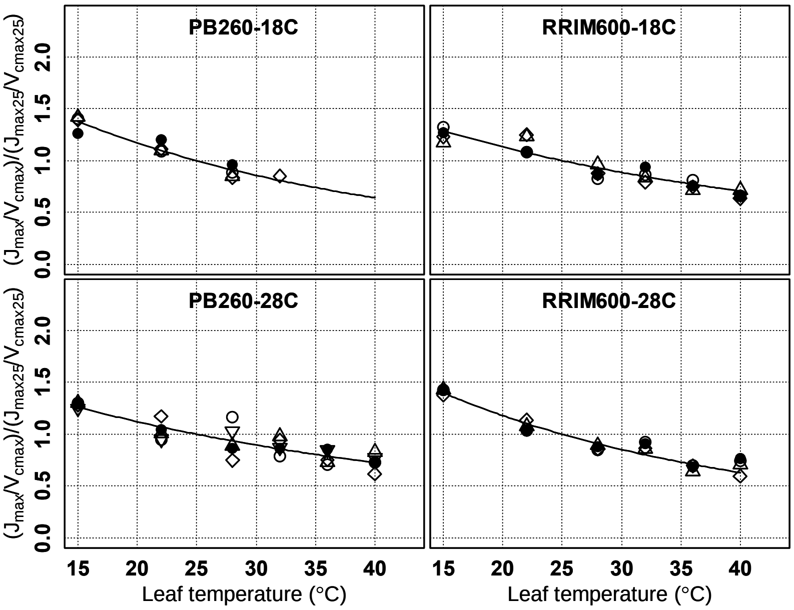


Figure 10 Temperature response of the ratio J_{max} to V_{cmax} (J_{max}/ V_{cmax}) (expressed as J_{max}/V_{cmax} to J_{max25} /V_{cmax25}) in PB 260 acclimated to 28 °C**.** Different symbols represent different leaves.

The balance between RuBP carboxylation and regeneration (J_{max}/V_{cmax})

The temperature response of J_{max}/V_{cmax} of PB 260 and RRIM 600 acclimated to 18 and 28 °C is shown in Figure 10. The ratio J_{max}/V_{cmax} decreased with increasing measurement temperature. The ratio of J_{max25} to V_{cmax25} increased significantly with decreasing growth temperature in one individual (Figure 11). The ratio J_{max} (25 °C) to V_{cmax} (25 °C) of RRIM 600 acclimated to 18 °C (1.91) was lower than plant acclimated to 28 °C (1.72) significantly. Whereas this ratio of PB 260 acclimated to

18 and 28 °C (1.95 and 1.86, respectively) were not significantly different (Figure 11).

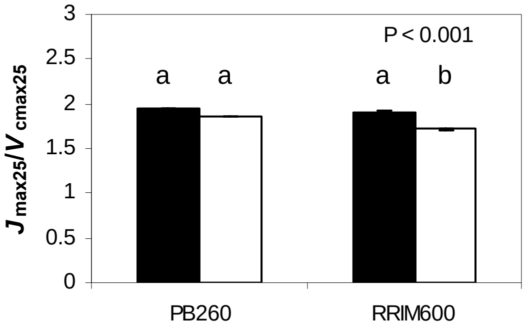


Figure 11 Mean values of the ratio J_{max25} to V_{cmax25} in rubber saplings acclimated to 18 °C (closed columns) and 28 °C (open columns). Bars represent ± SE (n = 4-5). Different letters above the column indicate statistical difference between different growth temperatures of the same clone. Significant levels: $P<0.001$.

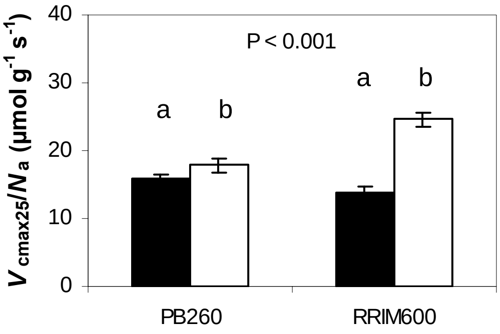
The mean values of the parameters describing the photosynthetic capacity averaging from both PB 260 and RRIM 600 grown at the same temperature are shown in Table 3.

Table 3 Mean values + SE (n = 4-5) of the parameters describing temperature responses of leaf photosynthesis in PB 260 and RRIM 600 grown at different temperature. Different superscript letters of values of same clone indicate statistical difference between different growth temperatures. Abbreviation: A, net CO_2 assimilation rate; A/C_i , CO_2 response curve; E_aV, activation energy for V_{cmax}; E_{aJ}, activation energy for J_{max}; ; J_{max25}, J_{max} at 25 °C; SE, standard error; T_{growth}, growth temperature; V_{cmax25}, V_{cmax} at 25 °C.

Parameter	PB 260		RRIM 600	
T_{growth} (˚C)	18	28	18	28
$V_{cmax\ 25}$	30.7 ±_2.9^{a}	49.0 ±_4.5^{b}	21.5 ±_2.2^{a}	38.7 ±_3.6^{b}
E_{aV}	54.5 ±_2.0^{a}	70.9 ±_1.7^{b}	65.6 ±_2.0^{a}	66.2 ±_1.8^{a}
$J_{max\ 25}$	59.8 ±_18.3^{a}	89.3 ±_19.0^{b}	41.7 ±_7.6^{a}	65.6 ±_12.0^{b}
E_{aJ}	32.6 ±_3.3^{a}	55.2 ±_2.0^{b}	45.9 ±_1.7^{a}	46.0 ±_1.8^{a}
J_{max25}/V_{max25}	1.95 ±_0.008^{a}	1.86 ±_0.005^{a}	1.91 ±_0.006^{a}	1.72 ±_0.006^{b}

Table 4 Mean values ± SE (n = 4-5) of leaf characteristics of PB 260 and RRIM 600 grown at different temperatures. Abbreviations: C, carbon; C_a, carbon per leaf area; LMA, leaf mass per area; N , nitrogen; N_a, nitrogen per area; SE, standard error; T_{growt}h, growth temperature (°C).

Rubber	PB 260		RRIM 600	
T_{growth} (°C)	18	28	18	28
LA (cm^2)	111.1 ± 23.3 ^{a}	103.7 ± 19.4 ^{a}	45.5 ± 6.2 ^{a}	125.6 ± 27.4 ^{b}
DM (g)	0.76 ± 0.2 ^{a}	0.62 ± 0.1 ^{a}	0.27 ± 0 ^{a}	0.55 ± 0.1 ^{b}
LMA (g m^{-2})	68.5 ± 4.8 ^{a}	60.5 ± 5.0 ^{b}	59.6 ± 1.3 ^{a}	43.8 ± 2.8 ^{b}
SPAD	45.9 ± 2.4 ^{a}	61.2 ± 3.1 ^{b}	37.3 ± 2.0 ^{a}	50.0 ± 2.8 ^{b}
N (%)	2.84 ^{a}	4.56 ± 0.2 ^{b}	2.60 ^{a}	3.61 ± 0.1 ^{b}
N_a (g m^{-2})	1.94 ± 0.1 ^{a}	2.76 ± 0.2 ^{b}	1.55 ± 0 ^{a}	1.58 ± 0.1 ^{a}
C (%)	47.4 ± 0.7 ^{a}	48.5 ± 0.5 ^{a}	47.3 ± 0.5 ^{a}	47.9 ± 0.5 ^{a}
C_a (g m^{-2})	32.5 ± 2.3 ^{a}	29.4 ± 2.5 ^{a}	28.2 ± 0.8 ^{a}	20.9 ± 1.2 ^{b}
V_{cmax25}/N_a	15.8 ± 0.6 ^{a}	17.8 ± 1.0 ^{b}	13.8 ± 1.0 ^{a}	24.5 ± 1.1 ^{b}
J_{max25}/N_a	30.8 ± 1.1 ^{a}	32.4 ± 1.2 ^{a}	26.9 ± 0.8 ^{a}	41.5 ± 4.8 ^{b}



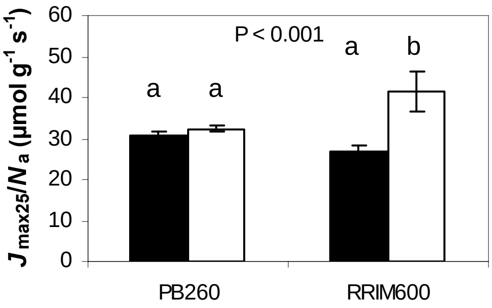


Figure 12 Mean values of photosynthetic nitrogen use efficiency (PNUE) expressed as the ratio of photosynthetic capacity (V_{cmax25} and J_{max25}) to nitrogen content per unit leaf area (N_a) (V_{cmax25}/N_a and J_{max25}/N_a) in rubber saplings acclimated to 18 °C (closed columns) and 28 °C (open columns). Bars represent ± SE (n = 4-5). Different letters above the column indicate statistical difference between different growth temperatures of the same clone. Significant levels: $P<0.001$.

Table 4 shows the effect of growth temperature (T_{growth}) on leaf characteristics at the end of the experiment. Leaves of individuals acclimated to 18 °C turned out to be less green and displayed smaller chlorophyll content per unit leaf area as indicated by the SPAD values. Leaf mass per area (LMA) was higher in plants grown at 18 °C than at 28 °C. Nitrogen content (N_m) was significantly lower in plants grown at 18 °C.

Photosynthetic nitrogen use efficiency (PNUE) expressed as V_{cmax25}/N_a and J_{max25}/N_a, where N_a is the leaf nitrogen content per unit leaf area, significantly decreased in plants grown at 18 °C (Figure 12).

Part II Effect of leaf age and season on photosynthesis of 2-year-old field-grown rubber trees.

Experiment 4.1 Effect of leaf age on photosynthesis in rainy season (October)

In October 2006, rubber trees clone RRIM 600, PB 260 and RRIT 251 had 5, 4 and 4 age classes of leaves (whorls), respectively in the canopy. The youngest whorl (W4, W4 and W5 of PB 260, RRIT 251 and RRIM 600, respectively) were not fully mature like other whorls. Leaf area and dry weight were lower in the oldest whorls for the 3 clones. (Figure 13 and 14).

Leaf mass per area (LMA) decreased significantly with whorl position in PB 260, but not clearly in RRIM 600 and RRIT 251, except that the youngest whorl always had the lowest LMA (Figure 15). Leaf greenness as indicated by SPAD of the youngest whorl (W5) had a lower SPAD than other whorls in RRIM 600 (Figure 16). This showed that this whorl was not fully mature.

Nitrogen content per unit leaf mass (N_m, %, Figure 17), increased with whorl position (decreased with leaf age) for each clone except in W5 in RRIM 600, whereas nitrogen per leaf area (N_a, g m^{-2}) did not change that clearly with whorl position (Figure 18).

Maximum net assimilation rate (A_{max}) increased with whorl position (leaf age) for each clone (Figure 19). The relationship between A_{max} and whorl position differed among clones. A_{max} differed significantly between each whorl (W1 to W4) in PB 260, whereas there was no difference between youngest whorls in RRIM 600 (W3-W5) and in RRIT 251 (W3-W4). Nevertheless, there was a decreasing trend in A_{max} with leaf age for the three clones. Leaves of the oldest whorl (W1) ranged 15% (RRIT 251) to 32% (RRIM 600) of youngest whorl (W4).

Nitrogen use efficiency (A_{max}/N_a) changed in the same way as A_{max} except in W5 in RRIM 600 (Figure 20).

Parameters of the biochemical model of leaf photosynthesis, i.e. the maximum rate of electron transport or RuBP regeneration (J_{max}) and the maximum rate of Rubisco activity or RuBP carboxylation (V_{cmax}), could not be estimated for the oldest leaf whorl (W1) in the 3 clones as stomatal conductance was too low, less than 0.04 mol m^{-2} s^{-1} and A/C_i curves obtained from these leaves were not reliable.
As activation energy (E_a) has not been estimated from the curve of temperature response of V_{cmax} and J_{max} for clone RRIT 251, the average E_a between RRIM 600 and PB 260 (Experiment 3, Table 3) was used to normalize these parameters for the three clones. Table 5 shows that, although normalized V_{cmax} and J_{max} at 25°C were much lower than parameters estimated at ambient temperatures, use of clone-specific or averaged E_a had little influence.

Table 5 Ratio of normalized photosynthetic parameters (V_{cmax25} and J_{max25}) to parameters estimated at ambient temperature (V_{cmax} and J_{max}) in RRIM 600 and PB 260 in October 2006. Clone E_a: clone-specific activation energy. Avg E_a : activation energy averaged for the 2 clones.

	V_{cmax25}/V_{cmax}		J_{max25}/J_{max}	
Clone	Clone E_a	Avg E_a	Clone E_a	Avg E_a
PB 260	0.53	0.54	0.61	0.64
RRIM 600	0.58	0.57	0.72	0.69

V_{cmax25} and J_{max25} increased significantly with whorl position (Figure 21 and 22), except that the youngest whorl was sometimes lower than the one which just was older. J_{max25} of oldest measured leaves (W2) was 62% (PB 260) to 79% (RRIM 600) of maximum (W3 or W4). V_{cmax25} of oldest measured leaves (W2) was 83% (RRIM 600) to 88% (RRIT 251) of maximum (W3 or W4). Hence changes of both parameters were in a much lower range than that of A_{max}. The ratio J_{max25}/V_{cmax25} was lower in the oldest leaves (W2).

Stomatal conductance (g_s) decreased strongly with leaf age (increased with whorl position), except that W5 of RRIM 600 had lower g_s than W3 and W4 (Figure 23). The very low g_s in W1 (oldest leaves) did not allow to estimate J_{max} and V_{cmax}. Variations in g_s with leaf whorl position were of the same magnitude than variations of A_{max}, then larger than variations in J_{max25} and V_{cmax25}.

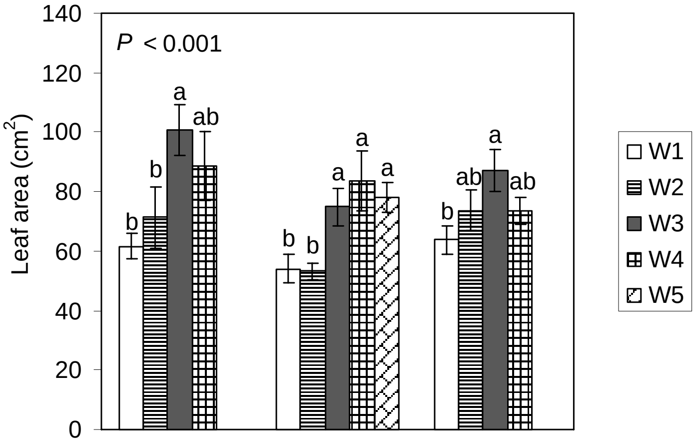


Figure 13 Effect of whorl position on leaf area in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

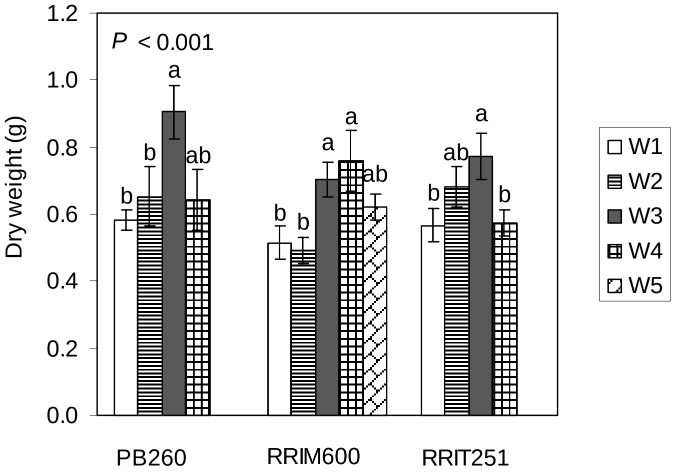


Figure 14 Effect of whorl position on dry weight in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

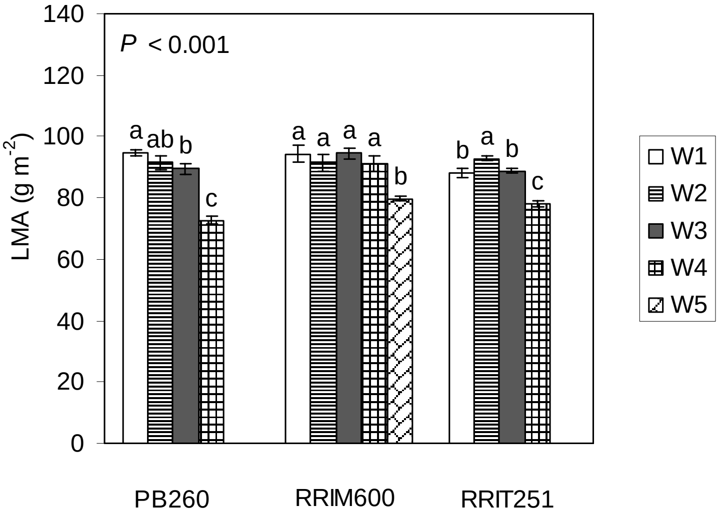


Figure 15 Effect of whorl position on leaf mass per area (LMA) in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

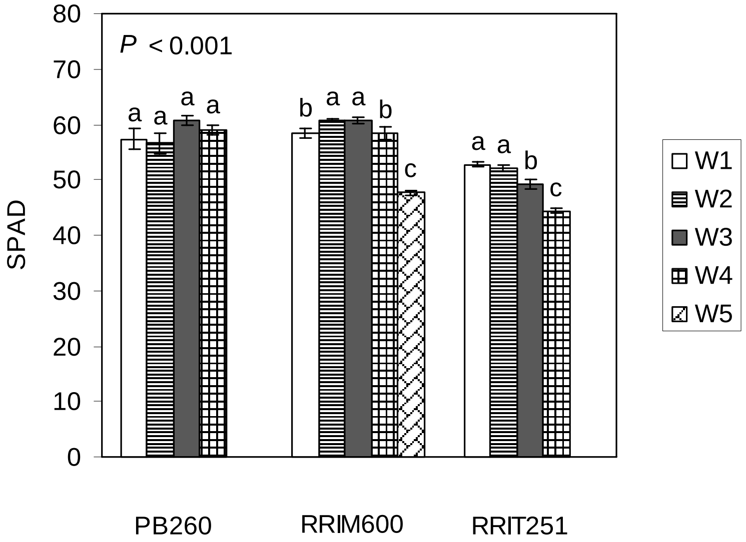


Figure 16 Effect of whorl position on SPAD in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

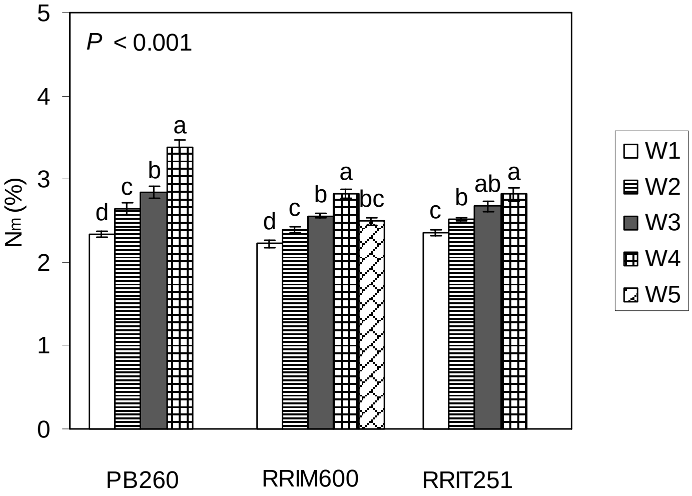


Figure 17 Effect of whorl position on nitrogen per unit mass (N_m, %) in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

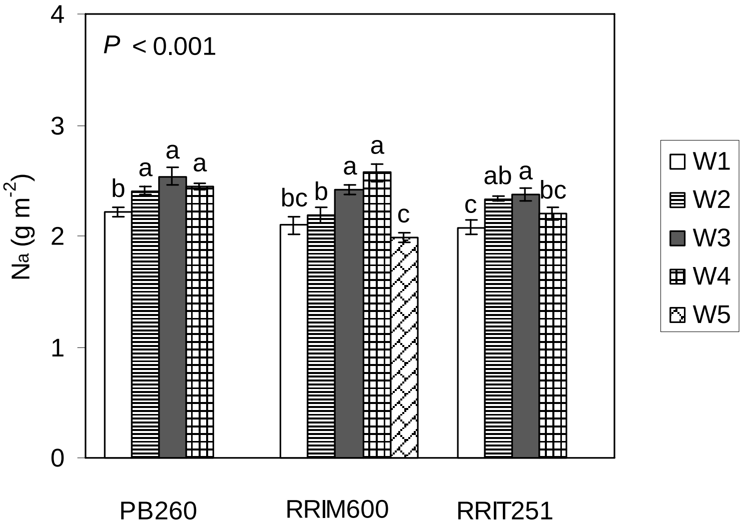


Figure 18 Effect of whorl position on nitrogen per unit leaf area (N_a, g m^{-1}) in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

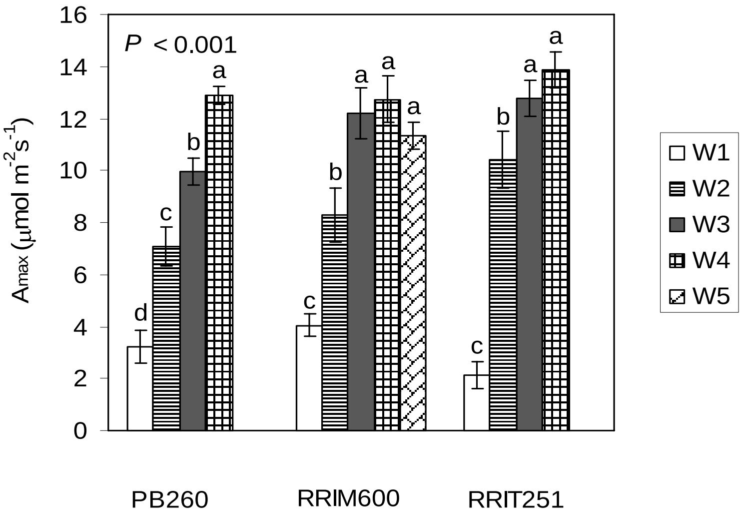


Figure 19 Effect of whorl position on net CO_2 assimilation rate (A_{max}) in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

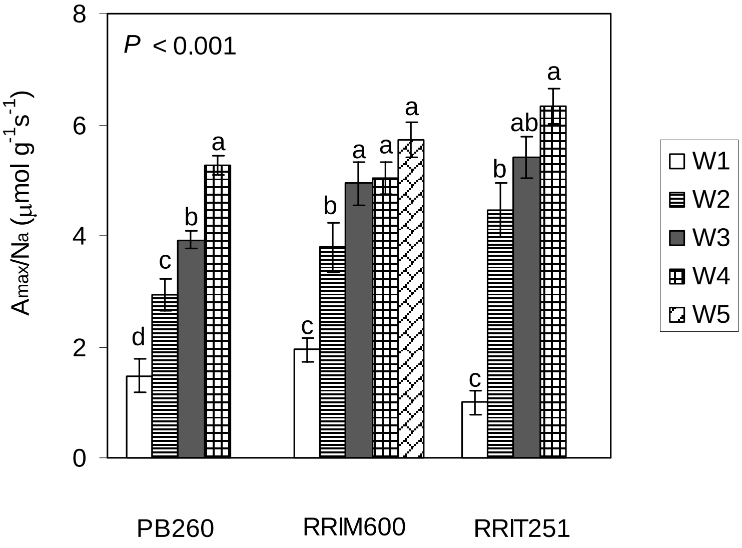


Figure 20 Effect of whorl position on photosynthetic nitrogen use efficiency (*A*/N_a, µmol g^{-1} s^{-1}) in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

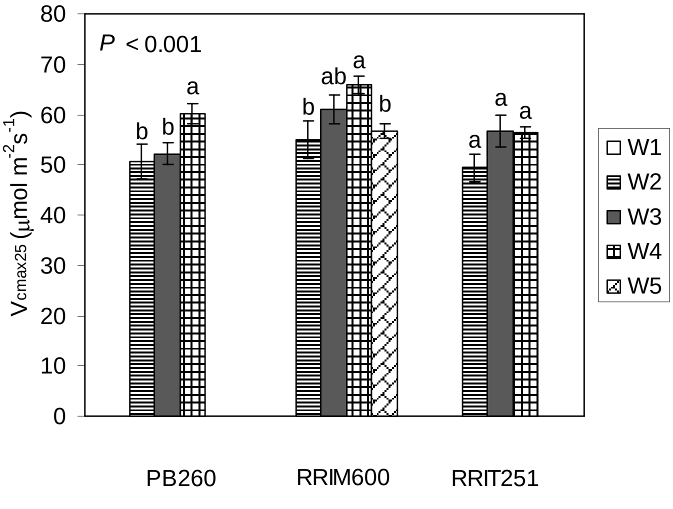


Figure 21 Effect of whorl position on maximum carboxylation rate (V_{cmax}) at a reference temperature of 25 °C in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

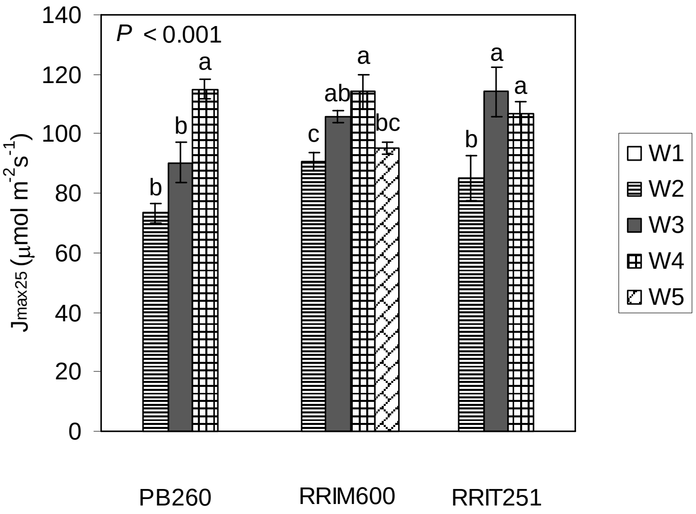


Figure 22 Effect of whorl position on maximum electron transport rate (J_{max}) at a reference temperature of 25 °C in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

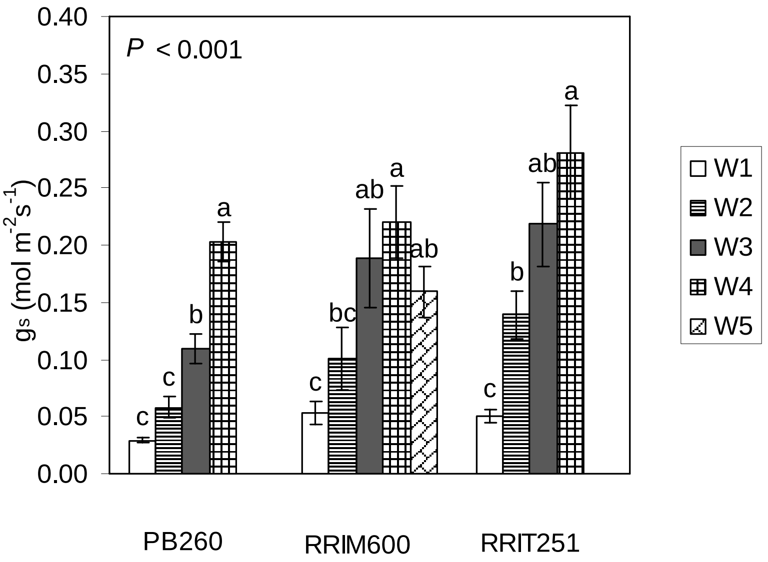


Figure 23 Effect of whorl position on stomatal conductance (g_s) at a reference temperature of 25 °C in 3 clones of 2-year-old rubber trees. Means (n=7), standard error of mean (vertical bars).

Experiment 4.2 Effect of season on photosynthesis of field-grown rubber trees.

The number of rainy days, amount of rainfall, evaporation pitch and mean air temperature in 2006 and 2007 are shown in Table 6. The climatic condition in October 2006, December 2006 and March-April 2007 were different.

Table 6 Climate conditions in Chachoengsao rubber research center, year 2006 and 2007.

Year	Month	Number of rainy day	Rain (mm)	Evap Pitch (mm)	Mean Air Temp (ºC)
2006	January	0	0	162	17.8
	February	2	31	159	28.6
	March	9	101	173	29.6
	April	7	86	157	29.5
	May	21	221	156	29.0
	June	12	138	151	29.0
	July	15	194	154	28.7
	August	15	195	149	28.3
	September	22	404	115	27.9
	October	15	164	140	27.8
	November	2	9	143	28.1
	December	0	0	156	25.6

Table 6 (Continued)

Year	Month	Number of rainy day	Rain (mm)	Evap Pitch (mm)	Mean Air Temp (ºC)
2007	January	2	0.4	162.4	26.4
	February	5	23.5	140.5	27.4
	March	7	155	178.1	29.8
	April	10	248.3	151.1	29.5
	May	22	259.8	146.6	28.7
	June	12	115.2	147.3	29.6
	July	20	195.7	145.1	28.4
	August	13	58.6	144.6	32.1
	September	18	205.7	130.3	28.4
	October	12	76.8	121.2	27.7
	November	3	22.8	137.5	25.5
	December	0	0	146.1	26.7

Shoot development

In April, there was only one whorl (W1) in each clone, as complete leaf shed occurred in February. Budbreak and shoot elongation occurred just after, followed by leaf development and elongation. This first whorl was about 2 months old in April and did not last until measurements in December in the three clones. Clone RRIM 600 and PB 260 had a 5th whorl in October and December respectively, whereas RRIT 251 developed 4 whorls only.

Net CO_2 assimilation rate (A_{max}) and nitrogen content

In the first whorl (W1) A_{max} decreased between April and October in the 3 clones (Figure 24). RRIT 251 had the highest A_{max} in April (despite a lower N content), but the lowest in October. A_{max} of W1 in April was exactly the same as A_{max} of W2 in October in the 3 clones. For the same whorl position, A_{max} decreased largely between October and December, whereas nitrogen content (N_a) varied much less and in different ways according to whorl and clone (Figure 25). Consequently, nitrogen use efficiency (A_{max}/N_a, PNUE) decreased a lot between October and December in every whorl x clone combination (Figure 26).

Patterns were different between clones. In PB 260, A_{max} increased with whorl position (decreased with whorl age) in December as in October. A_{max} in December ranged 9% (W2) to 22% (W4) of A_{max} October. In RRIM 600 the oldest (W2) and the youngest (W5) whorls declined more than the median whorls. A_{max} in December reached only 5 % of A_{max} in October in W5, whereas it reached 35 % in W3 (Figure 24). RRIT 251 had a higher A_{max} than the other clones in December and without differences between whorls at that date. Consequently, oldest whorls declined less than youngest whorl between October and December. In this clone, A_{max} in December reached 43% and 30% of October value in W2 and W4 respectively.

Changes in N content seemed as much related to whorl position than to season. Particularly in RRIM 600 the pattern related to whorls was the same in December than in October. There was a clearer increase in N with whorl position in December, as well as a declining trend (in W1, W2 and W3) for the same position between October and December. Thus, N content tended to decrease with leaf age (Figure 25). Consequently, nitrogen use efficiency (A_{max}/N_a) was lower in December too (Figure 26). A_{max} decreased much more than V_{cmax25} between October and December.

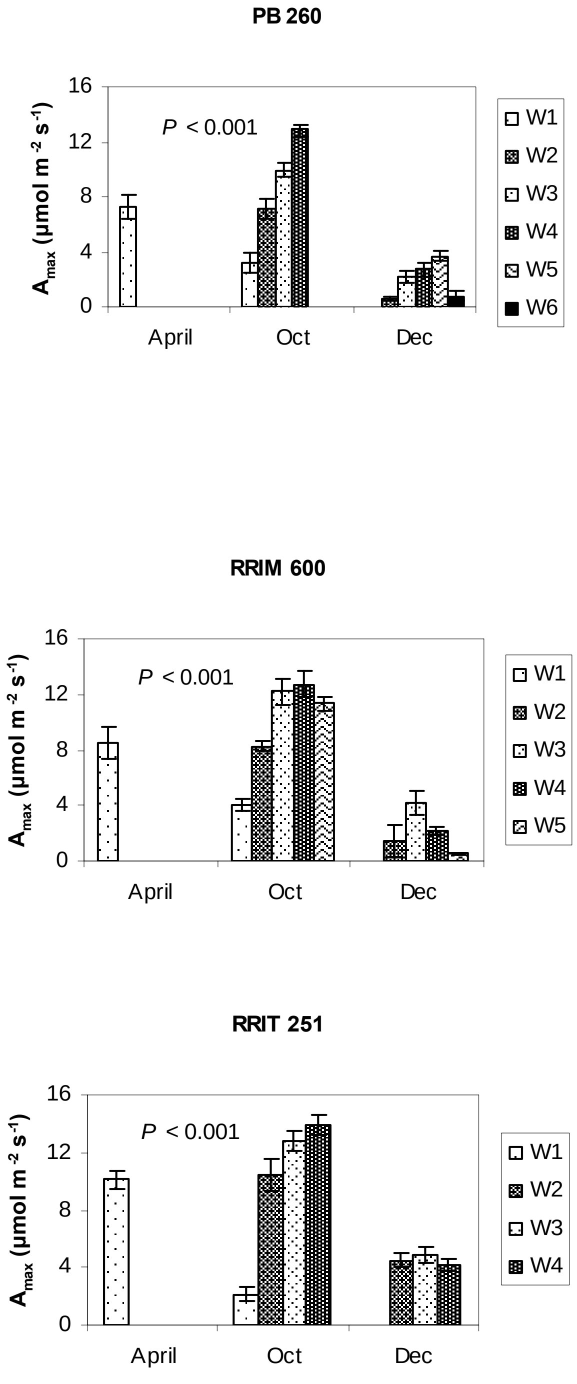
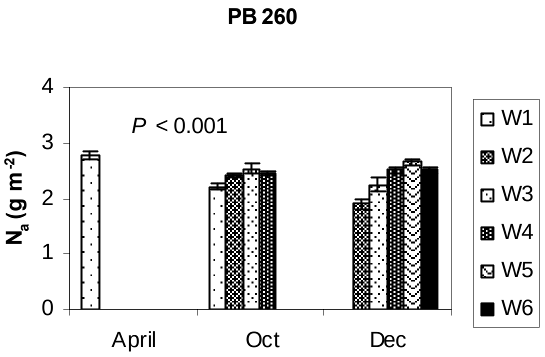
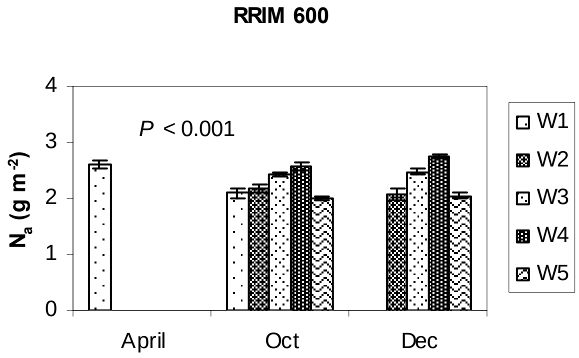


Figure 24 Net CO_2 assimilation rate in April 2007, October 2006 and December 2006. PB 260 (left), RRIM 600 (center), RRIT 251 (right). Means (n=7-16), standard error of mean (vertical bars).





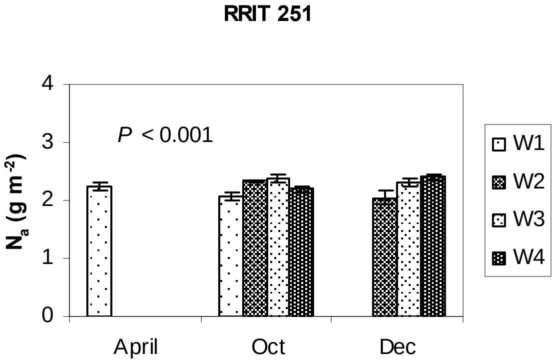


Figure 25 Nitrogen content per unit leaf area g m^{-2}) in April 2007, October 2006 and December 2006. PB 260 (left), RRIM 600 (center), RRIT 251 (right). Means (n=7-16), standard error of mean (vertical bars).

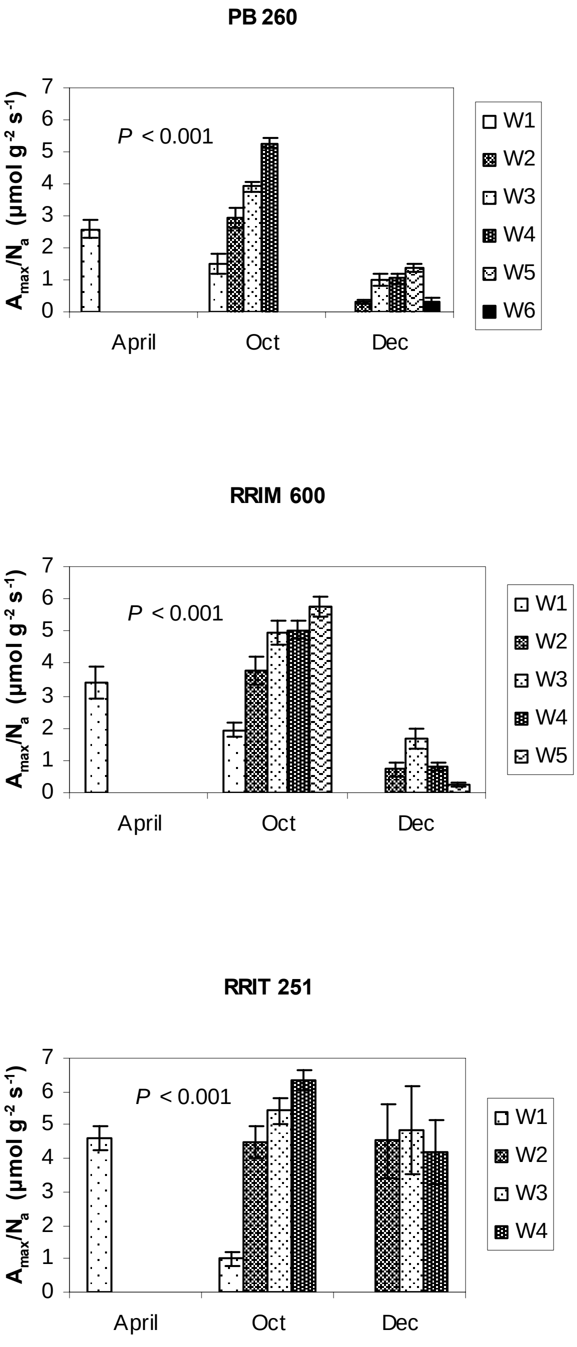


Figure 26 Photosynthetic nitrogen use efficiency (PNUE = A_{max}/N_a) in April 2007, October 2006 and December 2006. PB 260 (left), RRIM 600 (center), RRIT 251 (right). Means (n=7-16), standard error of mean (vertical bars).

Parameters of photosynthesis (V_{cmax} and J_{max})

In April, maximum rate of carboxylation (V_{cmax25}) was lower in PB 260, whereas maximum electron transport rate (J_{max25}) was not different between clones (Figure 27). It was not possible to measure V_{cmax} and J_{max} in W1 in October, to assess age effect in this first whorl. When W1 in April was compared to W2 in October (similar leaf age in different whorl), V_{cmax25} had the same clone positioning but with slightly lower rates in October (W2) than April (W1). J_{max25} was also lower in W2 in October than in W1 in April but clone positioning also changed. PB 260 was lower than the two others in October (W2), whereas there was no clonal difference in April (W1).

In December, due to excessively low stomatal conductance in many cases, complete A/C_i curves could be performed only in one clone, RRIM 600. Both A_c and A_j parts (full A/C_i curves) were obtained in most leaves in this clone, except some leaves for which only A_c part (a portion of A/C_i curve) was obtained. Thus V_{cmax} or J_{max}, or both could be estimated in some leaves. Results are shown for whorl 3 (W3), with parameters normalized for temperature at 25 ºC, which allowed to compare seasonal effect without impact of temperature (Figure 28). V_{cmax25} did not change between October and December, whereas J_{max25} decreased, but not in the same range as A_{max}. J_{max25}/V_{cmax25} tended to be lower in December, but the difference with October was not significant.

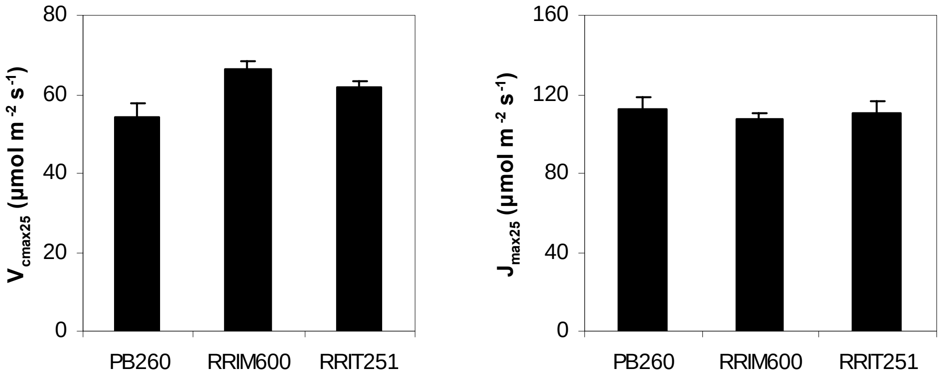


Figure 27 Maximum carboxylation rate (V_{cmax25}, left) and maximum electron transport rate (J_{max25}, right) of first whorl (W1) in PB 260, RRIM 600 and RRIT 251 in April 2007. Means (n=13-16), standard error of mean (vertical bars).

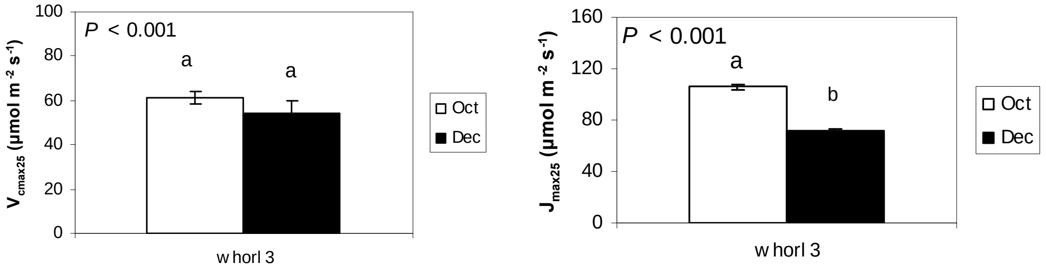


Figure 28 Maximum carboxylation rate (V_{cmax}) at a reference temperature of 25 °C (left) and maximum electron transport rate (J_{max}) at a reference temperature of 25 °C (right) of RRIM 600 in October and December 2006. Means (n=8-12), standard error of mean (vertical bars).

Discussion

Part I Photosynthesis in potted-rubber saplings

Temperature acclimation period

Acclimation of photosynthesis to growth temperature in the present study was studied by assessing acclimation of optimal temperature of net CO_2 assimilation rate and of photosynthetic capacity at a reference temperature of 25 °C (V_{cmax25} and J_{max25}), temperature responses of V_{cmax} and J_{max} (described by activation energy of V_{cmax} and J_{max} and optimal temperature), and the balance between RuBP carboxylation and regeneration (the ratio of J_{max} to V_{cmax}). Acclimation of photosynthesis may occur at different paces; the slowest processes occurring over days and implying changes in nitrogen allocation to photosynthesis and particularly to Rubisco, changes in the composition of thylakoid membranes that might result in increased stability at high temperature and enhanced optimal temperature for electron transport, as well as changes in Rubisco activation (Berry and Björkman, 1980; Sage and Kubien, 2007; Ow *et al.*, 2008). Most studies reported that acclimation of the photosynthetic apparatus may require a period of days or weeks (Berry and Björkman, 1980, and references therein), but some studies evidenced a rapid acclimation within 24 hours (Slatyer and Morrow, 1977, and references therein). The duration for temperature acclimation of at least three weeks in the present study was therefore sufficient for investigating the difference of response of photosynthesis to temperature resulted from the temperature acclimation in rubber saplings.

In addition, rubber leaves in this study were already fully expanded could acclimate to growth temperature. This suggests that the mature leaves have the ability to acclimate to changing growth temperature which is consistent with the report of Berry and Björkman, 1980.

Temperature response and acclimation of net CO_2 assimilation and photosynthetic capacity

In general, optimal temperature for net CO_2 assimilation (A) is around 30 °C for tropical species (Mason *et al.*, 2001). In this study, the optimal temperature for A of rubber saplings grown in the nursery and in a growth chamber at 28 °C was in the range 27-33 °C reported in earlier studies (Rao *et al.*, 1998, and references therein).

The rate of A declined below and above optimal temperature observed in this study has also been observed in many previous studies (Yamasaki *et al.*, 2002; Ambrosio *et al.*, 2006; Warren and Dreyer, 2006). The decline of A below and above optimal temperature could be explained by many reasons. At low temperatures, RuBP regeneration through the limitation of electron transport capacity is limited. The maximum activity of Calvin cycle enzymes such as fructose-1,6-bisphosphate and sedoheptulose-1,7-bisphosphate could also be inhibited (Ambrosio *et al.*, 2006, and references therein). Furthermore, end product limitation and orthophosphate (P_i) recycling to the chloroplast may significantly contribute to photosynthesis reduction at below optimal temperature and also above optimal temperature (Hendrickson *et al.*, 2004 and references therein).

Above optimal temperature, reasons for the decline of A are multiple. It is known that the deactivation of Rubisco limits photosynthesis directly at higher temperatures (Law and Crafts-Brandner, 1999; Salvucci *et al.*, 2001; Sharkey, 2005). The increase of the ratio of oxygenase to carboxylase activity of Rubisco (Jordan and Ogren, 1984) and the increase of the O_2/CO_2 solubility ratio (Ku and Edwards, 1977) result in a larger photorespiration and inhibition of A under moderately elevated temperatures. It has been suggested that photorespiration increases with temperature faster than photosynthesis does (Sharkey, 2005, and references therein). Recently, Kubien and Sage (2008) showed that decreasing of photosynthesis above optimal temperature resulted from RuBP regeneration limitation rather than of the capacity of Rubisco activase to maintain high Rubisco activation state. Moderately elevated temperatures also induce decreases in stomatal conductance, and in turn net photosynthesis, through a decrease of intercellular CO_2 concentration (C_i) decreases when temperature increases (Warren and Dreyer, 2006).

In the present study, V_{cmax25} and J_{max25} differed between plants acclimated during a month at 18 and 28 °C, and were larger in the latter case. This result is in agreement with earlier ones (Makino *et al.*, 1994; Medlyn *et al.*, 2002*b*; Warren, 2008). Unfortunately, the initial values of photosynthetic capacity had not been assessed before transferring the plants to the temperature treatments. Nevertheless, it is interesting that the observed changes were correlated with increased nitrogen, which is consistent with the study of Walcroft *et al.* (1997). As V_{cmax25} increased as nitrogen increased, this suggests that Rubisco concentration increased in plants acclimated to 28 °C. It is suggested that the Rubisco kinetics and the Rubisco activation state could acclimate to the growth temperature (Salvucci and Crafts-Brandner, 2004; Yamori *et al.*, 2006). It is well known that the photosynthetic capacity of leaves is related to the nitrogen content (Walcroft *et al.*, 1997; Medlyn *et al.*, 2002*b*) as V_{cmax} depends on the amount of Rubisco protein in the leaf and J_{max} depends on the amount of thylakoid components (von Caemmerer, 2000). The dependence of photosynthetic capacity on leaf nitrogen varies among species (Hikosaka *et al.*, 1998, and references therein). In our case, not only did the absolute values of V_{cmax} and J_{max} increase, but photosynthetic capacity per unit leaf nitrogen (PNUE, V_{cmax25}/N_a and J_{max25}/N_a) also increased. The difference in PNUE may be attributed to the difference in specific activity of Rubisco and nitrogen allocation to Rubisco (Hikosaka *et al.*, 1998; Warren and Dreyer, 2006). Since Rubisco is a key enzyme of photosynthesis, it has been shown that higher nitrogen allocation into Rubisco results in higher PNUE (Hikosaka *et al.*, 1998). The allocation of nitrogen is also correlated with leaf mass per area (LMA). In the present study, plants with larger LMA (grown at lower temperature, 18 °C) had lower photosynthetic capacity and PNUE, which consistent with the study in evergreen *Quercus* species (Takashima *et al.*, 2004). An increase in LMA may result from an accumulation of starch under low temperature (Usami *et al.*, 2001) because of decreased growth and decreased translocation. An increase in non structural carbohydrates may also cause an increase in LMA (Bertin *et al.*, 1999). It is also possible that leaves with larger LMA invested more nitrogen in cell wall. Plants grown at 18 °C allocated more nitrogen to cell wall than plants grown at 28 °C and resulted in increase in LMA and decrease in photosynthetic capacity and PNUE. It is hypothesized that there may be a trade-off in nitrogen partitioning between photosynthetic protein and structural protein in leaves (Takashima *et al*., 2004, and references therein).

The difference in PNUE of plants grown at different temperatures can also attributed to the difference in internal conductance to CO_2 transfer (g_i). The temperature is one factor of variation in g_i. Warren and Dreyer (2006) showed that g_i in deciduous oak (*Quercus canariensis*) increased with increasing temperature from 10 to 20 °C and did not increase from 20 to 35 °C, whereas Bernacchi (2002) reported that g_i in tobacco (*Nicotiana tabacum*) increased from 10 to 35 and 37.5 °C. However, g_i can affect the absolute values of photosynthetic capacity and the temperature response of photosynthesis (Warren and Dreyer, 2006).

In this study, optimal temperature (T_{opt}) for A differed at different growth temperatures. Rubber saplings grown at higher temperature displayed a higher optimal temperature for net CO_2 assimilation rate. This result is in agreement with previous results (Berry and Björkman, 1980; Ferra *et al*., 1989; Yamori *et al*., 2005; Hikosaka *et a*l., 2006). It has been suggested that the potential of acclimation of photosynthesis to temperature varies among species. For example, winter wheat (*Triticum aestivum* L. cv Norin No. 61) had an extremely high potential for temperature acclimation of photosynthesis, the optimal temperature for photosynthesis was 15 to 20 °C, 25 to 30 °C and around 35 °C in plants grown at 15, 25 and 35 °C, respectively (Yamasaki *et al*., 2002). Other species, cucumber *(Cucumis sativus* L.) shifted optimal temperature by 1 °C toward increasing growth temperature by 5 °C (Chermnykh and Kosobrukhov, 1987). In the present study, the shape coefficient of the temperature response (Ω) also differed among plants grown at different temperatures. Plants grown at lower temperature had narrower peak of temperature response curve of net CO_2 assimilation ((June *et al*., 2004). This suggests that growth temperature could affect the photosynthetic response to temperature and thus causes a change in the optimal temperature for photosynthesis (Medlyn *et al.*, 2002*a*; June *et al.*, 2004).

A change in the temperature response of V_{cmax} and J_{max} of plants grown at 18 or 28 °C did also clearly differ in this study. The optimal temperature for the two components of photosynthetic capacity (V_{cmax} and J_{max}) was above 36 °C and not far from 40 °C; this is in the range of values produced by Dreyer *et al.* (2001) which showed that the optimal temperature ranged between 35.9 and above 45 °C for V_{cmax} and between 31.7 and 43.3 °C for J_{max} in seedlings from seven temperate tree species. Other studies reported that the optimal temperature for V_{cmax} and J_{max} was 36.6 and 33.3 °C, respectively in silver fir seedlings (Robakowski *et al.*, 2002) and 34 and 33.3 °C, respectively in cork oak seedlings (Ghouil *et al.*, 2003).

Although the actual optimal temperature for the two components of photosynthetic capacity (V_{cmax} and J_{max}) was not adjusted due to the small number of points above optimum, activation energy of V_{cmax} (E_{aV}) and of J_{max} (E_{aJ}) was in one case higher in plants grown at higher temperature. This result is consistent with several studies (Hikosaka *et al.*, 1999; Onoda *et al.*, 2005*b*), whereas other study found no correlation of E_{aV} and E_{aJ} with growth temperature (Medlyn *et al.*, 2002*b*). In this study, the activation energy was also higher for V_{cmax} than for J_{max} resulting in a decrease in the ratio of J_{max} to V_{cmax} with increasing temperature.
In general, V_{cmax} and J_{max} are strongly correlated (Wullschleger, 1993). The balance between RuBP carboxylation and regeneration may change with growth temperature. The change in the ratio of J_{max25} to V_{cmax25} to growth temperature observed in this study has also been observed in many studies (Hikosaka *et al.*, 1999; Onoda *et al.*, 2005*a*; Yamori *et al.*, 2005). In contrast, Medlyn *et al.* (2002*b*) found no change in the ratio of J_{max} to V_{cmax} with growth temperature. However, it is suggested that change in the ratio of $J_{\mathrm{max}}/V_{\mathrm{cmax}}$ may involve in temperature acclimation of photosynthesis in some species (Onoda *et al.*, 2005*b*).

To our knowledge, similar experiments in the present study of temperature response of photosynthesis over a broad range of temperature and acclimation of photosynthesis to growth temperature have not previously been assessed in *Hevea brasiliensis* species. One other study only showed the different values of V_{cmax} and J_{max} estimated from A/C_{i} curves measured at 28 °C and nitrogen-use efficiency ($V_{\mathrm{cmax}}/N_{\mathrm{a}}$ and $J_{\mathrm{max}}/N_{\mathrm{a}}$) in different rubber clones (Sangsing *et al.*, 2004).

Part II Effect of leaf age and season on photosynthesis of 2-year-old field-grown rubber trees.

Significance of whorl position (leaf age)

Oldest and lowest whorls had a lower leaf area. This showed that differences between whorls could not be considered as related to leaf age only. There could be differences in morphology and physiology according to whorl position. However LMA was not different between leaves of all mature whorls, indicating that their morphology was likely similar and that they were neither thickening, nor decaying. Due to their different location within the canopy, low or high whorls could also have different light environment, interfering with the effect of leaf age. However, because of spacing and size of the trees at this age, light environment was not likely different between leaves, which could all be considered as sun-leaves. Moreover, similarity in A_{max} (Figure 21) between leaves of similar age but of different whorl and at different date (W1 in April and W2 in October) showed that most whorl effect could be accounted for by leaf age.

Moreover, even if a more precise follow-up would be necessary to really distinguish leaf age effect from whorl location effect, whorl position proved an appropriate parameter to classify leaves in the purpose of modeling canopy photosynthesis. This would be an easy-to-access parameter integrating both age and architectural effects. The result of the present study is in agreement with the study of Samsaddin and Impens (1979b,c) that net photosynthetic rate was affected by leaf age.

Whorl position effect

A_{max} of W3 to W5 was in the same range as A_{max} of young leaves in the pot experiments. There was a larger variability in A_{max} between whorls of the same clone than between clones for the same whorl position. Hence it is important to take whorl effect into account when determining parameters of photosynthesis models. Decrease in A_{max} was not linked to a decrease in nitrogen content and was of larger extent than decrease in J_{max} and V_{cmax}. The later changed few and, combined with stable N content, this could be interpreted as a rather stable level of Rubisco, allowing high capacity of RuBP carboxylation. As a whole, as photosynthetic capacity (V_{cmax}, $J_{max,}$ N content) seemed less affected than actual net photosynthetic activity (A_{max}), it is likely that another factor is involved to explain the decrease in efficiency of photosynthesis with leaf age (or at least whorl position). This could be internal conductance for CO_2 (g_i) in addition to stomatal conductance which was actually much affected.

J_{max} changed more than V_{cmax}, showing that regeneration of RuBP was more affected than its carboxylation. A_{max} and V_{cmax} decreased more and more regularly with leaf age in PB 260 than in other two clones. This may impact yearly net primary productivity of this clone.

Seasonal effect

Although in December V_{cmax} and J_{max} could be estimated for one whorl position in one clone only, Figure 21 showed that, during that time, seasonal effects could not be mistaken for age effects. First, changes in A_{max} between measurements dates were much larger than changes between whorls at the same date. Second, in December A_{max} decreased with whorl position (increased with leaf age) in RRIM 600, contrary to October. Thus, for example, A_{max} of whorl position 4 in December was not at all the same as A_{max} of whorl position 3 in October.

Clonal differences in December seemed related to phenology. The clone which did not develop a 5th whorl (RRIT 251) kept a higher A_{max} in October than the 2 others. In RRIM 600, decrease in A_{max} in December was huge, particularly in young and old whorls. Only leaves of whorl position 3 reached about 30 % of their A_{max} of October. They seemed more tolerant to adverse weather conditions.

As for effect of whorl position, decrease in A_{max} was not related to a decrease in N content, but in PNUE. N content seemed related to whorl position and leaf age, but not season. This means also that leaf of different ages (same whorl at different date) had sometimes the same N content. The later seems thus related to position within the canopy, and maybe, despite cover was not closed, to differences in light environment, known to influence N content.

V_{cmax} was not affected, whereas J_{max} decreased in December. Hence, lower photosynthesis seemed not related to carboxylation capacity of RuBP, which remained high. Although regeneration of RuBP (J_{max}) was lower, the change was not in the same range as change in A_{max}.

CONCLUSION

The present study suggests that rubber has a potential for acclimation of photosynthesis to growth temperature. This involved changes in photosynthetic capacity at a reference temperature (V_{cmax25} and J_{max25}) and to a lesser extent (and with less confidence) the temperature response of photosynthetic capacity, thus changing the temperature response of net CO_2 assimilation rate. Although rubber trees grown at 18 ˚C cannot maintain rates of net CO_2 assimilation rate, photosynthetic capacity and leaf nitrogen status close to those of rubber grown at 28 ˚C, they have the ability to succeed low temperature stress.

Photosynthetic parameters, A, V_{cmax}, J_{max} as well as photosynthetic nitrogen use efficiency ($A\mathrm{max}/N_{\mathrm{a}}$) varied seasonally and with leaf age. Therefore, leaf age and whorl position effects on photosynthesis should be taken into account in studies on canopy carbon exchange modelling in rubber trees.

With increasing concern about global climate change, the results from this study will be one of important data sets of tropical trees which will help us to understand better how photosynthesis respond and acclimate to temperature. This aspect would also be integrated to canopy carbon exchange modelling in rubber trees. Moreover, with the increased global demand for natural rubber resulting in the expansion of rubber plantations under unfavourable conditions with low temperatures, this study should bring insights for understanding photosynthesis limitation in rubber trees grown at low temperatures.

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