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Low temperature stress modifies the photochemical efficiency of a tropical tree species *Hevea brasiliensis*: effects of varying concentration of CO_2 and photon flux density

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Abstract

Two clones of *Hevea brasiliensis* (RRII 105 and PB 235) were grown for one year in two distinct agroclimatic locations (warmer and colder, W and C) in peninsular India. We simultaneously measured gas exchange and chlorophyll (Chl) fluorescence on fully mature intact leaves at different photosynthetic photon flux densities (PPFDs) and ambient CO_2 concentrations (C_a) and at constant ambient O_2 concentration (21 %). Net photosynthetic rate (P_N), apparent quantum yield for CO_2 assimilation (Φ_c), *in vivo* carboxylation efficiency (CE), and photosystem 2 quantum yield (Φ_{PS2}) were low in plants grown in C climate and these reductions were more predominant in RRII 105 than in PB 235 which was also reflected in their growth. We estimated in these clones the partitioning of photosynthetic electrons between CO_2 reduction (J_A) and processes other than CO_2 reduction (J^*) at low and high PPFDs and C_a. At high C_a (700 μmol mol^{-1}) most of the photosynthetic electrons were used for CO_2 assimilation and negligible amount went for other processes when PPFD was low (200–300 μmol m^{-2} s^{-1}) both in the C and W climates. But at high PPFD (900-1 100 μmol m^{-2} s^{-1}), J^* was appreciably high even at a high C_a. Hence at normal ambient C_a and high irradiance, electrons can be generated in the photosynthetic apparatus far in excess of what can be safely utilised for photosynthetic CO_2 reduction. However, at high C_a there was increased diversion of electrons to photosynthetic CO_2 reduction which resulted in improved photosynthetic parameters even in plants grown in C climate.

Additional key words: chlorophyll fluorescence; cold stress; partitioning of photosynthetic electron transport; photochemical efficiency; photoinhibition.

Introduction

Photochemical efficiency is commonly affected by stress conditions such as water deficit, low or high temperatures along with high irradiances (Aro *et al*. 1993, Long *et al*. 1994). There is more pronounced effect of low temperatures on the photochemical efficiency of plants of tropical origin than those from temperate climate (Allen and Ort 2001). *Hevea brasiliensis* is a tree species originally belonging to the tropical humid climate and thus being vulnerable to sub-optimal temperatures (Jacob *et al*. 1999, Alam and Jacob 2002, Devakumar *et al*. 2002, Ray *et al*. 2004). This cold susceptibility is mainly for the reduction in photochemical efficiency and increased

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Abbreviations: a.s.l. – above sea level; C – cold; C_a – ambient atmospheric CO_2 concentration; CE – *in vivo* carboxylation efficiency; C_i – leaf intercellular CO_2; F_0 – minimal fluorescence at dark adapted state; F_0'- minimal fluorescence obtained on far-red irradiation immediately after "actinic light" exposure; F_m – maximal fluorescence at dark adapted state; F_m' – maximal fluorescence under irradiation; F_v/F_m – ratio of variable to maximum fluorescence obtained after 20 min dark adaptation of the leaves; F_t – fluorescence at steady state; F_v'/F_m' – efficiency of excitation energy capture by open PS2 reaction centre; IRGA – infra-red gas analysis; J_A – rate of electron flow to CO_2 assimilation; J_T – rate of non-cyclic electron flow across PS2; J^* – rate of electron flow to processes other than CO_2 reduction; PAM – pulse amplitude modulated; $P_{max(Ci)}$ – C_i saturated CO_2 assimilation rate; $P_{max(PPFD)}$ – PPFD saturated CO_2 assimilation rate; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; q_N – non-photochemical quenching; q_P – photochemical quenching; ROS – reactive oxygen species; VPD – vapour pressure deficit; W – warm; Φ_c – apparent quantum yield for CO_2 assimilation; Φ_{PS2} – effective PS2 quantum yield.
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photoinhibition of photosynthesis (Huner *et al*. 1993, Fryer *et al*. 1998). When photochemical efficiency is reduced, photon energy absorbed by the plants becomes excess of its requirement for CO_2 reduction and over energisation of the thylakoid membrane occurs (Huner *et al*. 1993). The excess energy in the photosynthetic apparatus imparts various harmful effects leading to oxidative stress (Fryer *et al*. 1998).

Lowering the temperature generally reduces metabolic rates and can therefore limit the sinks for the absorbed excitation energy, particularly CO_2 fixation and other reductive processes including photorespiration (Huner *et al*. 1998). The highly reduced state of photosystem 2 (PS2) reaction centre can be considerably oxidised if CO_2 assimilation is increased and/or the excess energy is dissipated from the chloroplast as heat (Fryer *et al*. 1998). Therefore, allocation of photosynthetic electrons to CO_2 assimilation and other reductive processes becomes important under stress. We compared the photochemical efficiency of two *Hevea* clones grown in warmer and colder agroclimates and determined the partitioning of photosynthetic electrons for various processes at varying irradiances and ambient CO_2 concentrations (C_a). We also tested whether better photochemical efficiency was associated with better growth performance of the clones in colder (C) than warmer (W) climate and whether photochemical efficiency was considerably increased when measured at high C_a.

Materials and methods

Plants and locations: Two clones of *Hevea brasiliensis* namely PB 235 and RRII 105 were used in this experiment. The plants were grown in large polybag containers (0.75 m^3) filled with garden soil (equal mixture of red laterite soil, river sand, and farmyard manure) following all standard agronomic practices (Rubber Grower's Companion 1995) in two agroclimatically distinct locations. One location was the farms of Kerala Livestock Development Board (Indo-Swiss Project) located in Mattupetty (77°4´E, 10°5´N, 1 600 m a.s.l.), a hill station in the Western Ghats in the south Indian state of Kerala. The average temperature here is relatively cool and the winter (December to February) can be very cold with occasional ground frost. A parallel set of plants was kept as control at Rubber Research Institute of India, Kottayam (76°36´E, 9°32´N). Kottayam is on the plains (73 m a.s.l) about 150 km west of Mattupetty and about 15 km east of the Arabian Sea. Kottayam does not experience any low temperature stress.

The experiments were conducted during December 2000 when the age of the plants was eight months. The averages of daily minimum temperature for November and December 2000 in Mattupetty were 11.7 and 8.4 °C and the averages of daily maximum temperature were 23.2 and 23.9 °C, respectively. The corresponding means of daily minimum temperature in the plains of Kottayam were 23.2 and 20.7 °C while the means of daily maximum temperature were 32.0 and 31.9 °C, respectively. Temperature below 18 °C can be stressful and therefore affects the optimal growth of *Hevea* plants. Thus *Hevea* plants grown in Mattupetty experienced cold stress while their counterparts in Kottayam were free from it. Hence, Mattupetty is referred as C agroclimate and Kottayam as W agroclimate.

Simultaneous measurements of gas exchange and chlorophyll (Chl) fluorescence: All measurements were made on intact mature leaves attached with plants grown in the polybags. Net photosynthetic rate (P_N) and Chl fluorescence were measured simultaneously using a portable photosynthesis system (*LI 6400*, *LI-COR*, Logan, USA) and a pulse amplitude modulated Chl fluorometer (*PAM-2000*, *Walz*, Germany). A component (*GB-0161*) of the leaf chamber which had a provision to insert the fibre-optics probe of *PAM-2000* Chl fluorometer at about a 60^o angle from the surface of the leaf sample with the end of the fibre-optics probe supplied by *LI-COR* was used for the simultaneous measurement. It allowed for delivery of a saturation pulse of "actinic light" and detection of fluorescence signals. This enabled to simultaneously measure the gas exchange by *LI-6400* and the Chl fluorescence parameters in the *PAM-2000* fluorometer. We took care not to shade the leaf surface with the fibre optics of the fluorometer. This special leaf chamber component was also fitted with a PPFD sensor to record the irradiance on the leaf surface. As a source of "actinic light" for low PPFD, an external halogen lamp (*Osram*, Germany) fitted on a stand with an option to increase or decrease the irradiance *via* a 15-turn potentiometer (*2050-HB*, *Walz*, Germany) was used. For high PPFD, solar radiation was used as the "actinic light" source. To get a similar range of high PPFD from solar radiation, the measurements were conducted in the morning between 09:00 and 11:00 (local time). All these measurements were made at a leaf temperature of 25±0.5 °C, leaf-air VPD of 1.2–1.4 kPa, and at constant ambient oxygen concentration of 21 %. Different CO_2 concentrations (C_a) were generated inside the leaf chamber by using a CO_2 injector (*LI-6400-01*, *LI-COR*, Logan, USA).

The Chl fluorescence measurements were made following the techniques of Schreiber *et al*. (1998). CO_2 assimilation, stomatal conductance, and fluorescence (F_t) were constantly monitored to ensure that they reached steady state before a reading was taken. Maximal fluorescence under irradiation (F_m') was obtained by imposing a 1-s saturating flash to the leaf in order to reduce all the PS2 centres. Minimal fluorescence immediately after irradiation (F_0') was determined by covering the cuvette with a black cloth while a far-red radiation was simultaneously switched on to oxidise PS2 rapidly by

drawing electrons from PS2 to PS1. Effective PS2 quantum yield (Φ_{PS2}), efficiency of excitation energy capture by open PS2 reaction centre (F_v'/F_m'), and photochemical (q_P) and non-photochemical (q_N) quenching were calculated as follows: $\Phi_{PS2} = (F_m' - F_t/F_m')$, $F_v'/F_m' = (F_m' - F_0')/F_m'$, $q_P = (F_m' - F_t)/(F_m' - F_0')$, and $q_N = 1 - (F_m' - F_0')/(F_m - F_0)$ (Schreiber *et al*. 1998). The measurements were conducted both under low and high PPFD. The low PPFD was in the range of 200-300 µmol m^{-2} s^{-1} and the high PPFD was in the range of 900-1 100 µmol m^{-2} s^{-1}. Maximal fluorescence (F_m) and minimal fluorescence (F_0) of dark-adapted leaves were measured after dark-adapting the leaves for 20 min.

Photosynthetic response to PPFD was measured at a constant C_a of 350 µmol mol^{-1} by changing the irradiances using a LED source with a centre wavelength of 660–675 nm (*LI-6400-02*, *LI-COR*, Logan, USA) fitted to the leaf chamber of the photosynthesis system (*LI-6400*, *LI-COR*, Logan, USA). Each leaf was allowed to stabilise for about 5 min before taking the measurements. By plotting P_N *versus* PPFD we calculated the irradiance response curve. The apparent quantum yield of CO_2 fixation (Φ_c) was estimated as the slope of the linear portion of the PPFD response curve. PPFD saturated CO_2 assimilation rate $P_{max(PPFD)}$ and leaf intercellular CO_2 (C_i) saturated assimilation rate $P_{max(Ci)}$ were estimated from the irradiance response and CO_2 response curves, respectively. All the gas exchange parameters including C_i were calculated by the *LI-6400* software, which in essence follows the method of Caemmerer and Farquhar (1981).

Photosynthetic response to leaf C_i was measured at saturating PPFD by step changes of C_a in the measurement cuvette. Different CO_2 concentrations were generated inside the leaf chamber by using a CO_2 injector (*LI-6400-01*, *Li-COR*, USA). By plotting P_N *versus* C_i we calculated the CO_2 response curve. The *in vivo* carboxylation efficiency (CE) was estimated as the slope of the linear portion of CO_2 response curve.

Partitioning of photosynthetic electrons: The rate of non-cyclic electron flow across PS2 (J_T) was calculated from the Chl fluorescence as $J_T = PPFD \times 0.84 \times 0.5 \times \Phi_{PS2}$ where 0.84 is the fraction of the incident PPFD absorbed by the leaf in a C_3 species, and 0.5 is the fraction of PPFD absorbed by the light-harvesting complex of PS2 (Genty *et al*. 1989, Schreiber *et al*. 1998). The rates of electron flow to CO_2 assimilation (J_A) and to processes other than CO_2 reduction (J^*) were calculated as $J_A = 4(P_N + R_D)$ and $J^* = J_T - J_A$, respectively, following the technique of Cheng *et al*. (2001). R_D is day respiration under light from processes other than photorespiration, which was approximated as dark respiration for this experiment.

The P_N *versus* PPFD and P_N *versus* C_i curves were made in three plants from each clone. The other measurements were made in six to nine plants in each clone. Independent *t*-test was done to find the significance of the means.

Plant growth: Increment in girth (diameter) at 10 cm above the bud union and height of the plants were recorded in both the locations.

Results

CO_2 assimilation rate (P_N): $P_{max(PPFD)}$ was very small in C agroclimate and here the irradiance required to saturate P_N was lower than in W agroclimate (Fig. 1). Both P_N and the irradiance required to saturate P_N were much higher in PB 235 than in RRII 105 at C agroclimate, whereas at W agroclimate they were comparable. At saturating PPFD and increasing C_a, there was remarkable increase in P_N in both the clones at both the locations (Fig. 1). $P_{max(Ci)}$ was almost two fold that of $P_{max(PPFD)}$ at W agroclimate and was about three fold at C agroclimate (Fig. 2). Although Φ_c and *in vivo* CE of both the clones were less in C than W agroclimate, these were higher in PB 235 than RRII 105 in C agroclimate (Fig. 2).

PS2 quantum yield, excitation energy capture and dissipation: At C agroclimate, there was a 5 % reduction in maximum potential PS2 quantum yield (F_v/F_m in dark-adapted leaves) in comparison to the W agroclimate in clone PB 235 and 11 % in clone RRII 105. The average F_v/F_m values for PB 235 and RRII 105 in W agroclimate were 0.80 and 0.81, respectively. At saturating PPFD with increase in C_a there were increases in P_N and Φ_{PS2} in both the clones in both the locations (Fig. 3). In C agroclimate at a given PPFD, Φ_{PS2} was less in RRII 105 than in PB 235. Φ_{PS2} in RRII 105 was remarkably increased when measured in 700 µmol(CO_2) mol^{-1}. Similar trend was observed with q_P when measured at high PPFD (Figs. 4 and 5). Though clear difference in q_N between the two locations could not be detected in the present study, q_N decreased to some extent with increase in CO_2 at high PPFD in C agroclimate (Figs. 4 and 5). At low PPFD, F_v'/F_m' was significantly higher in PB 235 than RRII 105 in the C agroclimate (Fig. 6).

Partitioning of photosynthetic electrons: At a given PPFD, electron transport rate across PS2 (J_T) was less in C agroclimate than in the W one. At low PPFD both in the W and C locations, J^* was close to zero at C_a =700 µmol mol^{-1} indicating most of the electrons were used for CO_2 assimilation. But J^* was appreciably high when PPFD was high at C_a =700 µmol mol^{-1} (Fig. 7). In general, J^* was higher in C agroclimate than in W agroclimate (Fig. 7).

Plant growth: Growth performance of PB 235 was significantly higher than that of RRII 105 at C agroclimate

(Fig. 8). Girth and height of PB 235 were about 52 and 37 % higher than RRII 105, respectively, at C agroclimate.

Discussion

The differences in growth environments distinctly affected the photosynthetic potentials of *Hevea* plants. Low temperature had detrimental effects on their photosynthetic machinery. The low temperature induced effect was noticed at the levels of whole leaf gas exchange and of chloroplast photochemical activities.

Photochemical efficiency plays a key role in the response of plants to low temperature (Holaday *et al.* 1992, Baker 1994, Long *et al.* 1994). The PPFD response curve showed that both P_N and the PPFD required to saturate P_N were less in C agroclimate than in the W one. This means that the irradiance was highly in excess of the capacity of its utilisation by the *Hevea* plants in C agroclimate. At the same time, lower Φ_{PS2} in C agroclimate than in W agroclimate indicated decreased photochemical efficiency of the plants at the former location. The photochemical inhibition was reflected in the decrease in F_v/F_m, Φ_{PS2}, and q_P in C agroclimate in comparison to the W one. Since the W agroclimate is free from any low temperature stress, there was no such inhibition.

The excess photons that could not be used to drive useful photochemical reactions can cause metabolic

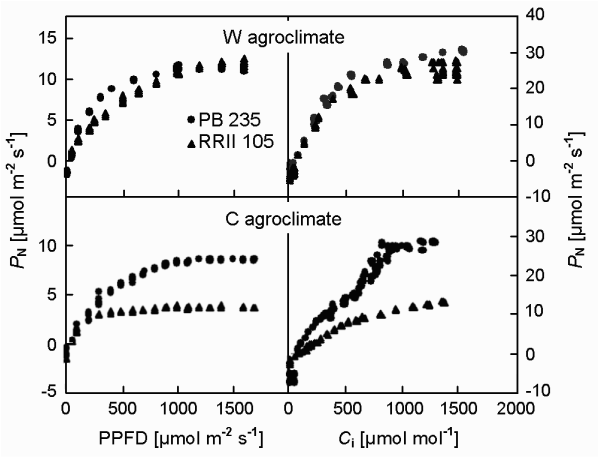

Fig. 1. Rates of net photosynthesis (P_N) *versus* photosynthetic photon flux density (PPFD) or intercellular CO_2 (C_i) in leaves of two clones of *Hevea* at two distinct agroclimatic locations (warmer and colder). n = 3.

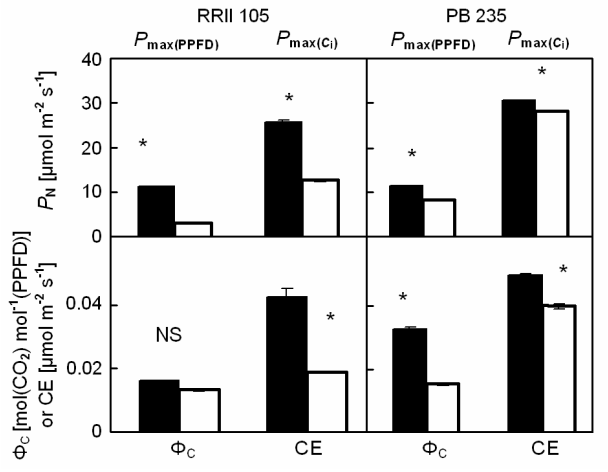

Fig. 2. Comparative photosynthetic parameters in two clones of *Hevea* at warmer and colder agroclimates. P_N, net photosynthetic rate [µmol(CO_2) m^{-2} s^{-1}], Φ_c, apparent quantum yield of CO_2 fixation [mol(CO_2) mol^{-1}(PPFD)], CE = *in vivo* carboxylation efficiency [µmol(CO_2) m^{-2} s^{-1}]. *Darkened bars* = W agroclimate, *open bars* = C agroclimate. Means±S.E., n = 3. $^*p$<0.05, NS = non-significant.

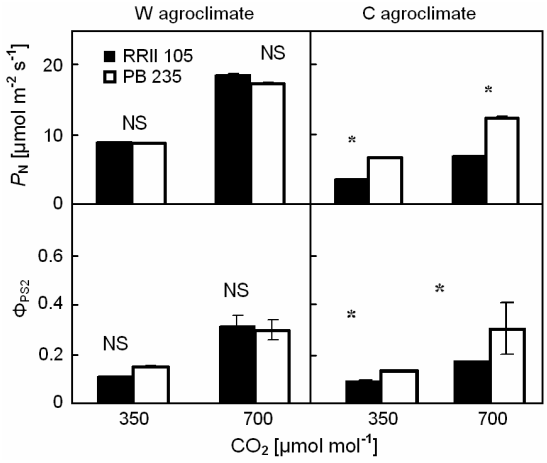

Fig. 3. Net photosynthetic rate (P_N) and photosystem 2 quantum efficiency (Φ_{PS2}) at saturating PPFD of two clones of *Hevea* at two different concentrations of CO_2 in warmer and colder agroclimates. Means±S.E., n = 6–9. $^*p$<0.05, NS = non-significant.

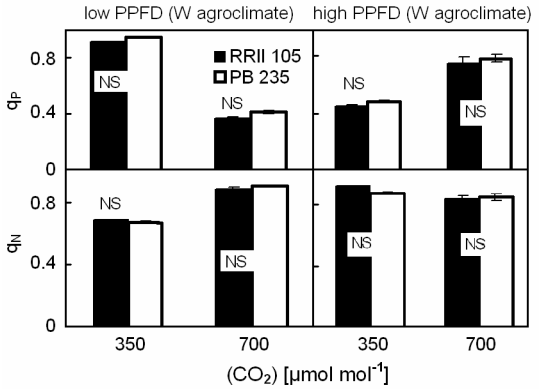

Fig. 4. Photosynthetic energy utilisation/dissipation through photochemical (q_P) and non-photochemical (q_N) quenching in the leaves of two clones of *Hevea* in warmer agroclimate. Low PPFD (200–300 µmol m^{-2} s^{-1}), high PPFD (900–1 100 µmol m^{-2} s^{-1}). Means±S.E., n = 6–9. NS = non-significant.

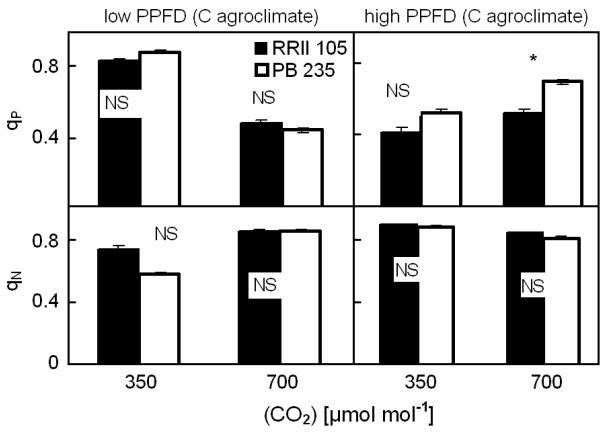

Fig. 5. Photosynthetic energy utilisation/dissipation through photochemical (q_P) and non-photochemical (q_N) quenching in the leaves of two clones of *Hevea* in colder agroclimate. Low PPFD (200–300 µmol m^{-2} s^{-1}), high PPFD (900–1 100 µmol m^{-2} s^{-1}). Means±S.E., n = 6–9. $^{*}p$<0.05, NS = non-significant.

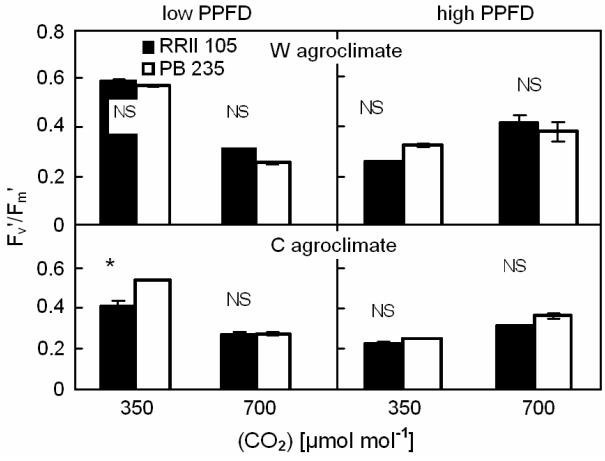

Fig. 6. Efficiency of excitation energy capture by open PS2 reaction centres (F_v'/F_m') in two *Hevea* clones in warmer and colder agroclimates. Low PPFD (200–300 µmol m^{-2} s^{-1}), high PPFD (900–1 100 µmol m^{-2} s^{-1}). Means±S.E., n = 6–9. $^{*}p$<0.05, NS = non-significant.

impairment (Barber and Andersson 1992, Adams *et al.* 1995). The excess photons would cause over-energisation of the thylakoid membranes leading to generation of excess electrons in the photosynthetic apparatus which can not be fully utilised for photosynthetic CO_2 reduction (Fryer *et al.* 1998). Over-production of photosynthetic electrons was associated with chilling injury in green leaves including *Hevea* leaves (Alam and Jacob 2002).

Most of the excess electrons that are not used for CO_2 reduction would be going into the alternative electron sinks, *e.g.* production of ROS including photorespiration (Fryer *et al.* 1998). In addition to this, low Φ_c in colder agroclimate compared to warmer agroclimate indicates that the plants in the former location remained less efficient in energy utilisation towards CO_2 assimilation. This may be the reason of higher flux of electrons to other reductive processes (J^*) in C agroclimate than in W agroclimate. This indicates that processes other than CO_2 assimilation can work as substantial alternative electron sink to protect PS2 from photodamage during exposure to sub-optimal temperature as suggested by Streb *et al.* (1998). Less photochemical efficiency and diversion of photosynthetic electrons from CO_2 assimilation might be responsible for the stunted growth of the plants in C agroclimate.

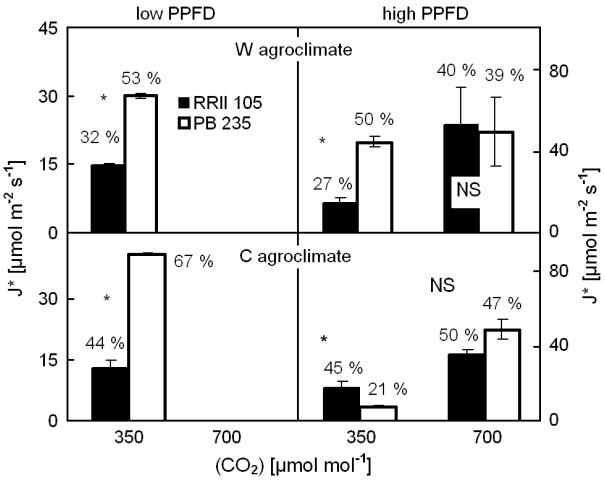

Fig. 7. Partitioning of photosynthetic electrons to processes other than CO_2 reduction (J^*) in two clones of *Hevea* at warmer and colder agroclimates. *The values over the bar* represent the percentage over the total photosynthetic electron transport across PS2 (J_T). Low PPFD (200–300 µmol m^{-2} s^{-1}), high PPFD (900–1 100 µmol m^{-2} s^{-1}). Means±S.E., n = 6–9. $^{*}p$<0.05, NS = non-significant.

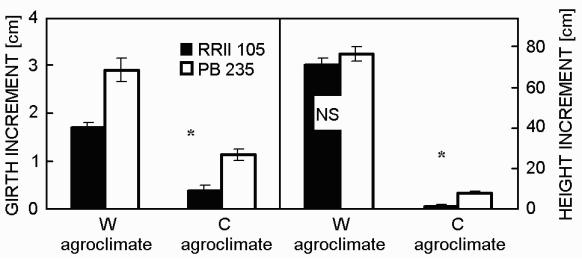

Fig. 8. Comparative growth performance of two *Hevea* clones at warmer and colder agroclimates. Means±S.E., n = 8. $^{*}p$<0.05, NS = non-significant.

Irrespective of the differences in growth environment, higher C_a in the ambient air during measurements improved the photochemical efficiency of the plants as reflected in higher P_N, Φ_{PS2}, and q_P. It implies that inhibition in photochemical efficiency due to sub-optimal temperatures could be improved considerably with higher CO_2 concentration by making more C_a available to photosynthesis. Our observations support the idea that plants grown under elevated CO_2 will have decreased intrinsic oxidative stress and higher C_a may lead to an increased metabolic flexibility to encounter such stress (Schwanz

et al. 1996, Devakumar and Jacob 1997, Polle *et al*. 1996, Azevedo *et al*. 1998, Morison and Lawlor 1999, Schwanz and Polle 2001).

We conclude that reductions in the photosynthetic functions in *Hevea* in the C agroclimate occurred mainly due to sub-optimal temperature. High PPFD further aggravated the effects of low temperature that down regulated PS2 activity. Maintenance of better photosynthetic performance in PB 235 was reflected in its better growth than RRII 105 in the C agroclimate. The decreased photochemical efficiency could be improved to a considerable extent when this was measured at higher C_a. This suggests that high C_a has stress ameliorating effects in *Hevea*. This may be attributed to the greater utilisation of photosynthetic electrons towards CO_2 reduction at high C_a. Our study also suggests that at high irradiance, electrons in the photosynthetic apparatus can be present far in excess of what can be safely utilised for photosynthetic CO_2 reduction.

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