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Leaf photosynthesis and chlorophyll fluorescence in four clones (400 series) of *Hevea brasiliensis* during low temperature period in Tripura

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Abstract

This present investigation was aimed at comparing the extent of variation in single leaf net photosynthetic rate (P_N) and its relative dependence on mesophyll capacity to fix carbon in four new clones of *Hevea brasiliensis* (RRII 414, RRII 422, RRII 429 and RRII 430) which were grown under the agro-climatic condition of Tripura. Photosynthesis and fluorescence parameters were measured during low temperature period. There were significant variations in P_N measured at various irradiance levels. The compensation irradiance (CI) and apparent quantum yield of CO_2 assimilation (Φc) calculated from P_N/PFD response curves showed significant variation among these four clones. Photosynthetic rate at saturated Ci (P_{maxCi}), *in vivo* carboxylation efficiency (CE) and CO_2 compensation concentration (Γ) calculated from P_N/Ci response curves showed significant variation. A strong positive correlation existed between P_N saturated with radiant energy (P_{sat}) and carboxylation efficiency (CE). A negative relationship between Γ and CE was observed in these clones. A clone with large P_{sat}, high CE and low Γ may be more biomass producer. Diurnal pattern of dark adapted Fv/Fm was also monitored during peak winter period to assess the extent of acclimation to low temperature. Results indicate that RRII 429 with high P_{sat}, high CE and low Γ showed relatively better acclimation compared to other clones. However, RRII 430 was also found to be promising at early growth stage.

Keywords: Chlorophyll fluorescence, *Hevea brasiliensis*, low temperature stress net photosynthesis

Introduction

Hevea brasiliensis, a forest tree which is indigenous to the tropical rain forests of Central and South America and the only source of natural rubber (NR). This tree had been domesticated as a plantation crop in various agro-climatic zones of the world (Archer and Audley 1973). In India, the cultivation of NR has not been confined only in Kerala, but it has spread over diversified agro-climatic conditions such as north eastern (NE) region of India. The major drawback for growing NR in NE region is the vulnerability of the this tree species to low winter temperature as it originally belongs to tropical humid climate (Jacob *et al.*, 1999, Alam and Jacob 2002, Ray *et al.*, 2004). Therefore, development of location specific clones has become a priority of crop improvement programmes in this crop. Apart from location specific clones, yet another dimension is to develop high yielding clones to further increasing the productivity of the crop.

There are many clones bred to suit different agro-climatic conditions. Rubber yield, biomass production, timber yield and many other physiological parameters do vary among the clones. Hence, physiological characterization of these newly arriving clones grown under prevailing low temperature stress condition in NE region is important in designing further breeding strategies.

Studying the yield or biomass production in a perennial tree crop like *Hevea* is highly tedious and time consuming. Any experimentation for studying the behaviour of certain physiological parameters which can effectively be used to assess relative performance any clone is always required. Significant difference in leaf photosynthetic parameters and fluorescence traits exists across the tree species (Nataraja 1991, Zipperlen and Press 1996, Alam *et al.*, 2005). In *Hevea*, clonal variation in photosynthesis has been observed (Samsuddin 1986, Dey *et al.*, 1995, Nugawela *et al.*, 1995). Detailed studies

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on leaf gas exchange and leaf chlorophyll fluorescence characteristics have been carried out in several tree species to understand the intrinsic capability of the species to withstand abiotic stress (Niinemets and Kull 2001, Ishida *et al.*, 2005). Under abiotic stress condition such as low temperature, net photosynthetic rate (P_N), apparent quantum yield for CO_2 assimilation (Φc), in vivo carboxylation efficiency (CE) and photosystem II (PSII) quantum yield (Φ_{PSII}) were studied in two clones of *Hevea* to investigate the acclimation strategies with respect to low temperature (Alam *et al.*, 2005). At the same time, assessment of photoprotection mechanisms under any abiotic stress condition has also been studied in many species through chlorophyll fluorescence. The extent of reduction in quantum efficiency of PSII as measured by variable to maximum chlorophyll fluorescence yield ratio (Fv/Fm) after dark adaptation of leaf is one of the most reliable parameters used to assess the capability of a species for photoprotection strategies under stress (Maxwell and Johnson 2000, Hendrickson *et al.*, 2004)

In the present study a detailed investigation was carried out to assess the photosynthetic capability to acclimate low temperature stress through *in situ* gas exchange and fluorescence measurements during winter period (December-January) in the newly introduced clones of 400 series namely, RRII 414, RRII 422, RRII 429 and RRII 430.

Materials and methods

Bud grafted plants of four *Hevea* clones namely RRII 414, RRII 422, RRII 429 and RRII 430 were planted in polybags filled with garden soil following standard agronomic practices in July 2006 and 2007. (Rubber grower's companion 2005) in Taranagar experimental farm located at Agartala, Tripura, northeast India (23°53′ N, 91°15′E, 20 m MSL). Experiments were conducted during December 2007- January 2008 (winter). Average minimum temperature recorded during this period was 12°C with a lowest minimum of 8.8 °C.

Photosynthesis measurements

In this experiment, variation in photosynthesis under different photosynthetically active radiation (PAR) incidental on the leaf lamina was measured. Photosynthetic rate (P_N) and related gas exchange parameters were recorded using an Infra red gas analyzer (IRGA) (CIRAS II, PP systems, USA).

P_N-PPFD response curves: Leaves of five individual plants of each clone were tagged at the beginning of each day for studying the response of various photosynthetic photon flux densities (PPFD) on photosynthetic rate (Photosynthetic rate, P_N versus PPFD response curve). A fully matured leaf of top most whorls was clamped in a standard 2.5 cm^2 broad leaf cuvettes. Light was provided by a red-blue light emitting diode (LED array) and ambient CO_2 partial pressure was maintained. The data logger was programmed in such way that it logged at 21 different light levels between 0-2000 µmole $m^{-2}s^{-1}$. The leaf was given 45 seconds to stabilize. The leaf temperature was tracked as ambient. The VPD was maintained less than 1 KPa.

P_N-Ci curves: P_N-Ci curves (Photosynthetic rate (P_N) plotted against intercellular CO_2 concentration) were generated using another leaflet of the same leaf on the same day of measuring P_N-PPFD response curves to estimate the potential photosynthetic capacity of *Hevea* plants under two contrasting seasons. Leaves were placed in the cuvettes at a CO_2 concentration ranging from 10-1000 ppm and illuminated at 1000 µmole $m^{-2}s^{-1}$PAR. Data in both response curves were calculated by using 'Photosyn Assistant' software, Dundee Scientific, UK. to arrive at different photosynthetic parameters.

Fluorescence parameters measurements

Chlorophyll fluorescence was measured using a portable pulse amplitude modulated fluorometer (FMS 2, Hansatech Ltd, UK). The leaves were first dark adapted for minimum of 20 minutes. The measuring light used was 0.1 µmol $m^{-2}s^{-1}$. The saturating light was applied by LED array with an intensity of 4000 µmol $m^{-2}s^{-1}$ for a short pulse. The baseline minimum fluorescence (Fo) and maximum fluorescence (Fm) were measured diurnally starting from 6 AM to 5:30 PM. The variable fluorescence (Fv = Fm-Fo) and ratio of Fv/Fm, photochemical efficiency of PS II were calculated (Maxwell *et al.*, 2000). Data were statistically analyzed and t-test was used to compare the clones.

Results and Discussion

Photosynthetic parameters

Photosynthetic rate at saturating PFD (P_{sat}) computed from PN/PPFD response curves ranged from 3.11 µmol CO_2 m^{-2} s^{-1} (RRII 414) to 7.60 µmol CO_2 m^{-2} s^{-1} (RRII 429). The maximum apparent quantum yield for CO_2 fixation (Φc) was recorded in RRII 429 and the minimum in RRII 414 (Table 1). Significant difference in compensation irradiance (CI) was also recorded ranging from 35 µmol $m^{-2}s^{-1}$ in RRII 429 to 134 µmol $m^{-2}s^{-1}$ in RRII 414 (Table 1). Genetic variability in photosynthetic rate at saturated PFD in *Hevea* was reported earlier by Nataraja and Jacob (1999). In this present investigation also, there was wide variation in P_{sat} (59%). In addition to that, significant variation in Φc was noticed. The total canopy photosynthesis and dry matter production was determined more by Φc than P_{sat} (Long, 1985). Significant positive correlation between Φc and rubber yield potential has been reported in *Hevea* (Nugawela *et al.*, 1995).

Significant differences were also found in the response of P_N to Ci (Fig. 1).The calculated value of photosynthetic rate (P_N) at saturating Ci (P_{maxCi}) ranged from 6.60 μmol $CO_2 m^{-2}s^{-1}$ in case of RRII 414 to 11.60 μmol CO_2 m^{-2} s^{-1} in case of RRII 430 under low temperature stress (Fig. 2). Among these clones, RRII 429 maintained maximum P_N of 6.93 when measured at Ca (350 ppm). The *in vivo* carboxylation efficiency (CE) alculated from P_N/Ci response curves was highest in RRII 430 and lowest in RRII 414. With respect to CO_2 compensation concentration (Γ) there was significant difference among these four *Hevea* clones. Leaf dark respiration rate (Rd) varied by 47% among the clones. The clone RRII 414 showed 1.46 μmol $CO_2 m^{-2}s^{-1}$ where as RRII 430 recorded 0.77 μmol $CO_2 m^{-2}s^{-1}$ (Table 1). Because of higher P_N at Ca (350 μmol $m^{-2}s^{-1}$) and high Φc, clone RRII 429 may be considered more adapted to prevailing agro climatic condition particularly low temperature stress compared to RRII 414 which has shown relatively less P_N and Φc. Low CI is beneficial for higher productivity in the shaded part of the canopy. The clone RRII 429 with relatively lower CI of 35 (μmol $m^{-2}s^{-1}$) may have advantage of having better mesophyll efficiency to fix carbon at low PFD.

Table 1. Net photosynthetic rate saturated with irradiance, P_{sat} [μmol (CO_2) $m^{-2}s^{-1}$], apparent quantum yield for CO_2 fixation, (Φc) [μmol(CO_2) mol^{-1}(PFD)], Compensation irradiance (CI), [μmol $m^{-2}s^{-1}$], Leaf dark respiration rate (Rd) [μmol (CO_2) m-2s-1]

Clones	P_{sat}	Φ_c	CI	Rd
RRII 414	3.11 (±0.235)	0.0108 (±0.002)	134 (±13.2)	1.46 (±0.17)
RRII 422	4.33 (±0.134)	0.0129 (±0.002)	80.1 (±17.0)	1.04 (±0.09)
RRII 429	7.60 (±0.255)	0.0365 (±0.004)	35.0 (±9.2)	0.86 (±0.14)
RRII 430	3.82 (±0.162)	0.0209 (±0.001)	60.2 (±15.0)	0.77 (±0.05)
CD (P=0.05)	1.12	0.029	9.82	0.02

In addition to that, there was a positive correlation between Psat and Φc (r^2=0.78, p<0.01)) observed among the clones. The carboxylation efficiency (CE) was negatively correlated with CO_2 compensation concentration (Γ) (r^2=0.58, p<0.01). Stomatal limitation to photosynthesis (Ls) was also significantly different among the clones. The clone RRII 414 and RRII 430 showed minimum and maximum stomatal limitation (Fig. 2). Variation in Γ very often indicates the variations in photorespiration which decreases the photosynthetic productivity by decreasing the CE (Morgan and Austin

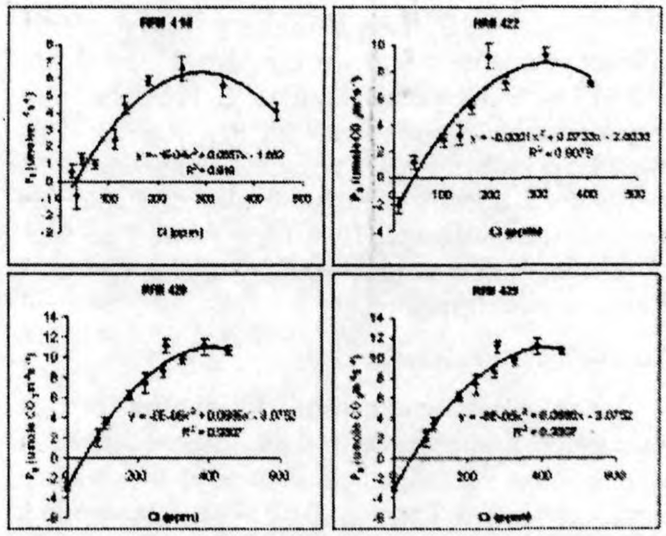

Fig. 1. Net photosynthetic rate/intercellular CO_2 concentration (Pn/Ci) response curves in four new Hevea clones.

1983). The results of the present experiment also revealed the similar relationship in *Hevea* clones under study (Fig. 2).

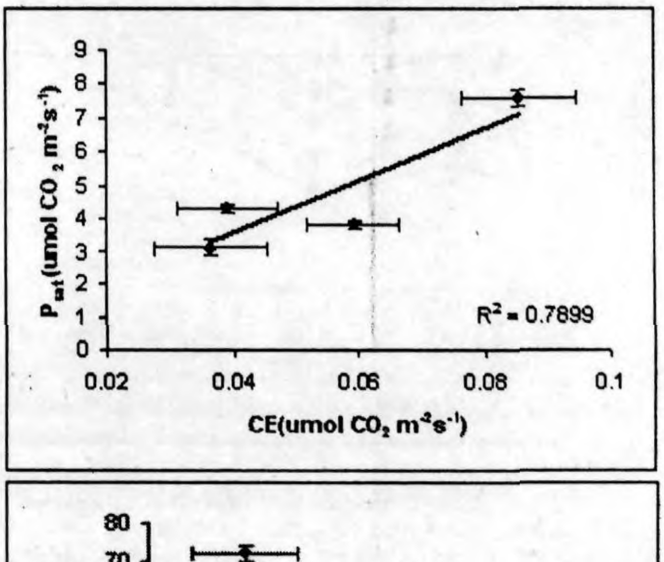

Fig. 2. Linear regression between (A) photosynthetic rate at saturated level of radiant energy (P_{sat}) and carboxylation efficiency (CE) (B) CO_2 compensation concentration (Co_2) and carboxylation efficiency (CE)

However, the variation in CE among four clones of *Hevea* under the present investigation, indicate that RRII 429 and RRII 430 with more CE are distinctly different compared to other two clones RRII 422 and RRII 414 which have relatively low CE. The CE showed strong positive correlation with P_{sat} (Fig. 2). This relationship is obvious as *in vivo* CE is a measure of the activity of the primary carboxylase enzyme, Rubisco in the leaves (Farquhar *et al.*, 1980, Long 1985). Therefore, CE is an index of mesophyll capacity of photosynthesis (Farquhar *et al.*, 1982).

Chlorophyll fluorescence

The measurements of diurnal pattern of chlorophyll fluorescence in dark adapted leaves of various clones indicated their adaptability to prevailing climatic conditions. The clone RRII 414 recorded greater decline in dark adapted Fv/Fm ratio through out the day which was significantly different from rest of the clones. The ratio was 0.642 at 6 am, then declined to 0.627 at 12 noon and the recovered to 0.762 at 5.30 pm. The clone RRII 430 recorded the dark adapted Fv/Fm ratio of 0.671

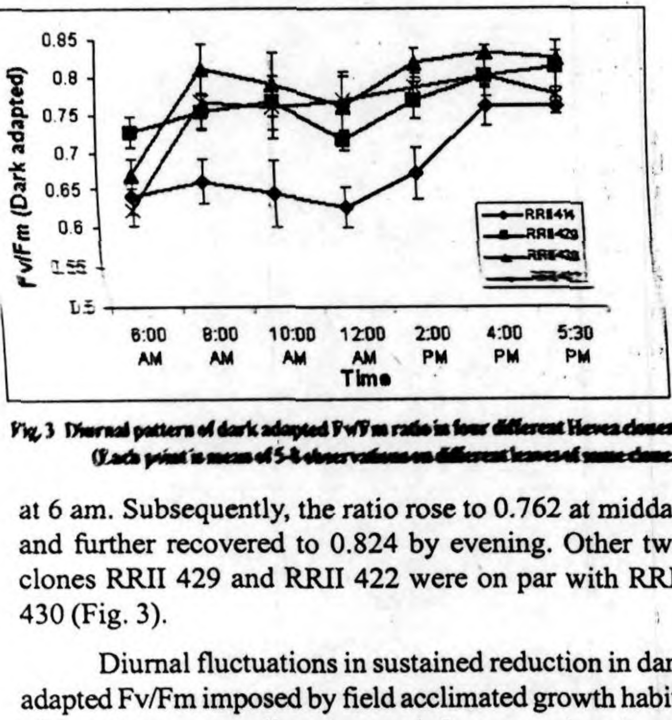

Fig. 3 Diurnal pattern of dark adapted Fv/Fm ratio in four different Hevea clones. (Each point is mean of 5-6 observations on different leaves of same clone.)

at 6 am. Subsequently, the ratio rose to 0.762 at midday and further recovered to 0.824 by evening. Other two clones RRII 429 and RRII 422 were on par with RRII 430 (Fig. 3).

Diurnal fluctuations in sustained reduction in dark adapted Fv/Fm imposed by field acclimated growth habits of the plants are well understood. In grape vine, it was studied to assess the sensitivity to chilling temperature particularly sensitivity of photosynthesis *per se* (Hendrickson *et al.*, 2004a).

Overall, the results indicate that RRII 429 with high P_{sat}, high CE and low Γ showed relatively better acclimation compared to RRII 414. However, RRII 430 also showed promising gas exchange and fluorescence traits under this agro-climatic condition indicating that this clone is also a better performer at early growth stage. Such physiological characterization of new clones is important to find out location specific suitability and also to refine further breeding strategies in crop improvement programme in *Hevea brasiliensis*.

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