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HIGHER LEAF AREA PER PLANT CAN COMPENSATE THE DECLINE IN NET PHOTOSYNTHETIC RATE IN MODERATELY SHADE GROWN SEEDLINGS OF *HEVEA BRASILIENSIS* IN LOW WINTER TEMPERATURE

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SUMMARY

Photosynthetic performance of *Hevea brasiliensis* seedlings at different light conditions was studied to assess their adaptability to low light levels. Seedlings were grown under full light as well as low light conditions (65% and 25% of incoming radiation). Leaf photosynthetic rate (P_N) was higher in full sunlight grown plants both during the first week of February (winter) and April (post-winter) than low light grown plants. Under severe low light condition (25% of incident radiation), the photosynthetic rate was as low as 2.0 µmole CO_2 m^{-2} s^{-1} in winter and 10.31 µmole CO_2 $m^{-2}s^{-1}$ in post winter period when measured at ambient light. In addition to that, photosynthetic rate (P_N) versus incident radiation (PFD) curves indicated that the light saturated photosynthetic rate in full sunlit plants was higher than shade grown plants. Chlorophyll fluorescence studies carried out during the winter season revealed that dark adapted Fv/Fm was higher in shade grown plants than open grown plants. This result indicated that low temperature-induced photo inhibition was severe in full sunlight grown plants than to shade grown plants which was reflected by low leaf chlorophyll content in open grown plants than shade grown plants. However, a small decline in leaf photosynthesis under 65% light condition was more than compensated by large increase (54%) in leaf area per plant leading to higher net canopy photosynthesis. Thus the plants grown under 65% light condition were taller and also had higher biomass than full sunlit plants during low temperature period. The increased biomass in *Hevea* seedlings at 65% light condition may be due to higher canopy photosynthesis.

Key words: **Canopy leaf area, chlorophyll content, low light, photosynthesis, shade acclimation**

INTRODUCTION

Hevea brasiliensis is the only latex-producing species that is commercially cultivated in a limited geographical area of the world to meet the global demand of natural rubber. Though this tree originated from the Amazonian forest of Brazil, it was initially grown in certain pockets of the sub-tropical regions of Southeast Asia, called as traditional rubber growing belt and later its cultivation was expanded to other regions with optimal climatic conditions. One such region with optimal climatic conditions for *Hevea* cultivation is the northeastern states of India. Climatic condition of this region such as low winter temperature along with high irradiance causes down- regulation of photosynthetic process known as photoinhibition. Photoinhibition occurs when irradiance is received in excess of that is required for carbon assimilation (Demming-Adams and Adams 1992, Osmond 1994). High irradiance can induce a decline in photosynthetic productivity in many crop species (Baker

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et al. 1994, Barth *et al.* 2001), including *Hevea* (Jacob *et al.* 1999, Senevirathna *et al.* 2003). Specific to *Hevea*, it was observed that high irradiance was detrimental for young plants when they are normally grown as monoculture plantation in Sri Lanka (Stirling *et al.* 2001). Previous studies suggested that intercropping enhances growth of young rubber plants (Rodrigo *et al.* 1997, 2001).

In northeast India young *Hevea* seedlings are usually exposed to potentially harmful irradiance often exceeding 1500 μmole $m^{-2}s^{-1}$ around midday during cold winter season in northeast India (Ray *et al.* 2004). Under such environmental conditions shade may be beneficial to improve photosynthetic efficiency in *Hevea* seedlings (Nair *et al.* 2002). However, it is unknown to what extent the photosynthetic machinery in *Hevea* can adapt shade conditions to result in better growth of the seedlings under the agro-climatic conditions of this region with the prevailing low temperature during the winter season. To investigate this, *Hevea* seedlings were grown at two different low light conditions namely partial shade (65% light) and deep shade (25% light) as well as full sunlight (100% light) condition for a period of six months including the winter season. In this experiment, the photosynthetic responses to low light conditions were studied.

MATERIALS AND METHODS

Experimental site and plant materials: The experiment was conducted on *Hevea* seedlings grown in a seedlings nursery at Taranagar research farm, regional research station of the Rubber Research Institute of India, Agartala, Tripura, India (91°15' E, 23°25' N: 30 m above MSL). Several nursery beds measuring 12m x 1m dimension were prepared with properly pulverised soil mixed with recommended proportion of sand and farm yard manure. Germinated seeds were placed at a depth of 1 inch of soil on the nursery bed with a spacing of 30 cm x 30 cm. Subsequently, seedlings were raised following recommended package and practices for *Hevea* seedling nursery (Rubber growers' companion 2008). Manual irrigation was provided on alternate days.

Experimental design: The experiment was conducted with three treatments i.e. 25%, 65% and full sunlight (100% light) with 5 replication plots for each treatment. Low light treatments were imposed on seedlings at 60 days after planting by placing a structure of net houses made up of shade-net having 75% and 35% light cut-off supplied by Tuflex India Inc, India. At the same site 5 replication plots were maintained under full sunlight condition as control.

Climate: The mean maximum and minimum air temperature for the winter period (November 2007-February 2008) was recorded as 28.7°C and 11.8°C. Mean relative humidity was about 75 ± 5.31%. A total of 43.5 mm rainfall was received during this period.

Photosynthesis measurements: *In situ* variations in photosynthesis under respective light conditions of each treatment were measured. The parameters such as P_N, g_s and other related gas exchange parameters and incident photon flux density (PFD) were recorded using an Infra red gas analyser (IRGA) (CIRAS II, PP systems, Amesbury, MA, USA) in five leaves of different plants in each replication plot. At the time of measurement, the leaves were exposed to average ambient temperature of 30.3°C and 77% relative humidity. The ambient CO_2 concentration was around 370 ppm and it was quite stable at the time of taking measurement.

P_N-PPFD response curves: Five leaves from different plants were tagged in each treatment for studying the P_N versus PFD response curve. A fully matured leaf of top most whorls was clamped in a standard 2.5 cm^2 broad leaf cuvette. Light was provided by red-blue light emitting diode (LED array) and ambient CO_2 partial pressure was maintained. The data logger was programmed in such a way that it was logged at 21 different light levels between 0-2000 mmol $m^{-2}s^{-1}$. For each light level the leaf was given 45 seconds to stabilise. The leaf temperature was tracked as ambient. The VPD inside the chamber was maintained less than 1 kPa.

The response of leaf net photosynthesis (P_N) to different light levels (PFD) was fit into a non-rectangular hyperbola model expressed as a quadratic equation by using Photosyn Assistant software (Richard Parsons, Dundee, UK.) The light compensation point was estimated from axis intercepts and the light saturated photosynthetic capacity (A_{max}) is the upper asymptote which was reached at the light saturation point.

Fluorescence parameter measurements: Chlorophyll fluorescence was measured using portable pulse amplitude modulated fluorometer (FMS 2, Hansatech Instruments Ltd, Norfolk, England) in all the leaves in which gas exchange measurements were taken. The leaves were first dark adapted for 30 minutes. The measuring light used was 0.1 µmole $m^{-2}s^{-1}$. The saturating light was applied by LED array with an intensity of 4000 µmole $m^{-2}s^{-1}$ for a short pulse. The baseline fluorescence (Fo) and maximum fluorescence (Fm) were measured starting from 8 am to 9.30 am. The variable fluorescence (Fv=Fm-Fo) and ratio of Fv/Fm was calculated. All data was statistically analysed and data on each treatment was compared using an independent t-test.

Growth measurements: Plant height and stem diameter at 10 cm height of 25 seedlings in each replication were recorded. There were 5 replications under each treatment. Growth measurements were taken once at the time of imposing the treatment (November) and finally at the end of the winter season (February). The increment in plant height and stem diameter was calculated by subtracting the initial value from the final one. Stem diameter was measured by using a digital Vernier caliper (0-200 mm). A representative sample of five seedlings was taken from each replication for estimating above-ground biomass per seedling at the end of the experiment (February). All leaves of each seedling were harvested and total leaf area was measured using a leaf area meter (LiCor 3000, Lincoln, NE). Leaves and stem of each seedling were oven-dried at 70°C till reaching constant dry weight. Total dry weight of each seedling was recorded. A surrogate measure of leaf chlorophyll content was taken with a Chlorophyll meter (CCM 200, Opti-Sciences, NY, USA) as Chlorophyll Content Index (CCI).

Analysis of data: Data was analysed by using regression techniques where ever applicable. Physiological parameters and growth traits were separated by one way ANOVA (Vassar Stats, NY, USA) followed by HSD test. All relationships were considered significant at $p<0.05$.

RESULTS

Growth: The plant growth measured as increment in stem height was maximum in partial shade (65% light) grown plants (9.0 cm) as compared to control (5.21 cm) and deep shade (25% light) grown plants (3.1 cm). No significant difference was observed in stem diameter (at 10 cm height) among the treatments (Table 2). However, partial shade grown plants recorded maximum above-ground dry biomass of 20.40 g/plant as compared to 17.14 g/plant in open plants and 15.75 g/plant in deep shade grown plants. Apart from that, the average total

Table 1a. Photosynthetic rate (P_N) (µmole CO_2 $m^{-2}s^{-1}$), Stomatal conductance g_s (mmolm$^{-2}s^{-1}$), intercellular CO_2 concentration Ci (ppm), mesophyll efficiency (Ci/g_s) and photosynthetically active radiation (PAR) (µmole $m^{-2}s^{-1}$) air temperature (C) and relative humidity (%) under three light conditions during winter (February).

Light environments	February (Winter)						
	P_N	g_s	*Ci*	Ci/g_s	*PAR*	*Temp*	*RH*
25% light	2.0	31.22	179	5.73	363	13.7	82
65% light	2.41	25.42	138	5.42	1083	14.3	77
Full light	3.35	34.75	133	3.82	1918	16.4	80
$CD_{(0.05)}$	0.66	ns	ns	-	92.6	ns	ns

Table 1b. Photosynthetic rate (P_N) (µmol CO_2 m^{-2} s^{-1}), Stomatal conductance g_s (mmole m^{-2} s^{-1}), intercellular CO_2 concentration Ci (ppm), mesophyll efficiency (Ci/g_s) and photosynthetically active radiation (PAR) (µmole m^{-2} s^{-1}) air temperature (C) and relative humidity (%) under three light conditions during post-winter (April).

Light environments	April (Post winter)						
	P_N	g_s	*Ci*	Ci/g_s	*PAR*	*Temp*	*RH*
25% light	4.28	79.3	208	2.62	178	29.8	77
65% light	10.31	82.2	94	1.14	656	31.2	75
Full light	12.67	95.1	60.7	0.63	1425	32.6	69
CD $_{(0.05)}$	1.62	ns	31.93	-	256	ns	ns

Table 2. Growth parameters such as plant height (cm), stem diameter (mm) at 10 cm height, total leaf area (cm^2 $plant^{-1}$), leaf chlorophyll content index (CCI) and total dry matter per seedling (g) under three light conditions during experimental period.

Light environments	Growth parameters				
	(Increments during experimental period)		Total leaf area / plant (cm^2)	Leaf Chlorophyll Content Index (CCI)	Total dry matter/ seedling (g)
	Plant height (cm)	Stem diameter (mm)(at 10 cm height)			
25% light	3.10	0.436	2281	47.81	15.75
65% light	9.0	0.508	2233	41.70	20.40
Full light	5.21	0.542	1476	34.20	17.14
CD $_{(0.05)}$	2.44	ns	641.7	5.85	2.9

leaf area per plant in deep shade and partial shade was 2281 and 2233 cm^2/plant respectively where as open plants recorded total leaf area of 1476 cm^2/plant.

The chlorophyll content indicated by the CCI values in low light plants was higher compared to open plants. The average CCI value in open plants was 34.2 whereas it was 41.70 and 47.81 in partial shade and deep shade grown plants respectively.

Leaf photosynthesis: The average PFD recorded in full sunlight condition was 1918 µmole $m^{-2}s^{-1}$ where as it was 1083 µmole $m^{-2}s^{-1}$ and 363 µmole $m^{-2}s^{-1}$ in partial shade and deep shade condition respectively during the winter period. Leaf photosynthetic rate in open plants during the winter season was 3.35 µmole CO_2 $m^{-2}s^{-1}$. Partial shade and deep shade grown plants recorded 2.41 µmole CO_2 $m^{-2}s^{-1}$ and 2.0 µmole CO_2 $m^{-2}s^{-1}$ respectively. There was no significant difference in stomatal conductance (g_s) and intercellular CO_2 (Ci) among the different light treatments. An inverse estimate of photosynthetic efficiency (Ci/gs) was low (3.82) in open plants as compared to low light plants (5.73 in deep shade grown plants and 5.42 in partial shade grown plants).

Among the above mentioned treatments, highest P_N was recorded in open light plants (12.67 µmole CO_2 $m^{-2}s^{-1}$), followed by partially shaded plants (10.31 µmol $CO_2m^{-2}s^{-1}$) and then deep shaded plants (4.28 µmole CO_2 $m^{-2}s^{-1}$).

Table 3. Gas exchange parameters derived from P_N/PFD response curves: A_{max} (μmole CO_2 $m^{-2}s^{-1}$), compensating irradiance (μmole $m^{-2}s^{-1}$), light saturating estimate (μmole $m^{-2}s^{-1}$) and fluorescence measurements (Fv/Fm) under three light conditions during experimental period.

Gas exchange parameters derived from P_N/PPFD response curves and fluorescence measurements	Light environments			
	25% light	65% light	Full light	$CD_{(0.05)}$
A_{max} (light saturated)	6.17	6.42	6.80	0.32
Compensating Irradiance (CI)	49.1	55.9	66.1	13.4
Light saturating estimate	186.3	230.3	368.3	24.8
Dark adapted Fv/Fm	0.821	0.802	0.773	0.02

Light response curve (P_N/PPFD curves): Gas exchange parameters derived from P_N/PFD curves revealed that light saturated photosynthesis (A_{max}) was recorded as 6.8 μmol CO_2 $m^{-2}s^{-1}$ in full sunlight grown plants and it was 6.42 μmole CO_2 $m^{-2}s^{-1}$ under partial shade and 6.17 μmole CO_2 $m^{-2}s^{-1}$ under deep shade conditions.

The light saturation for photosynthesis was attained at low PPFD of 186.3 μmol e$m^{-2}s^{-1}$ in deep shaded plants as compared to 230.3 and 368.3 μmole $m^{-2}s^{-1}$ in partially shaded plants and open plants respectively. The photosynthetic compensating irradiance (CI) was 66.1 μmole $m^{-2}s^{-1}$ in open plants followed by 55.91 μmole $m^{-2}s^{-1}$ in partially shaded plants and 49.11 μmole $m^{-2}s^{-1}$ in deep shaded plants.

Dark Fv/Fm: The ratio of variable fluorescence (Fv) maximal fluorescence (Fm) under dark adapted condition (Fv/Fm) was 0.821 in deep shaded plants. Full sunlight and partial shade grown plants showed reduced Fv/Fm ratio of 0.773 and 0.802, respectively.

DISCUSSION

Maximisation of light harvesting is one of the acclimation strategies of plants growing in light limited condition. In the present investigation, shade grown plants showed more chlorophyll content than open plants indicating an adaptive mechanism of maximising light harvesting systems through synthesising more chlorophyll (Mae 1997, Mielka and Schaffer 2010).

Full sun irradiance is not always beneficial for plants, particularly when they are grown under any environmental stress conditions such as low temperature, drought etc. Photosynthetic activity in young *Hevea* seedlings was low during the month of February than April (Ray *et al.* 2008). Further, the declining trend of A_{max} values in shaded plants during the winter period indicates low photosynthetic activity in *Hevea* under low light conditions in other plant species also as indicated by Valladares (2005). Higher leaf photosynthetic rate in open plants in comparison to low light grown plants was also previously observed in rubber plants (Nugawela *et al.* 1995).

Chlorophyll fluorescence studies carried out during the winter season revealed that dark adapted ratio of Fv/Fm was higher in shade grown plants than open grown plants. This result shows that low temperature-induced photoinhibition was more severe in open grown plants than to low light grown plants (Senevirathna *et al.* 2003). Similar results were also observed in rubber in previous studies that confirms photoinhibition in rubber under subtropical field condition at winter temperature (Ray *et al.* 2008). Photoinhibition reduces net carbon gain more severely at higher PPFD than in low PPFD. This was also supported by another study that daily reduction in carbon gain by 8% in open leaves of *Quercus coccifera* L. compared to 3% in shaded leaves (Werner *et al.* 2001).

In the present study, though full sunlight grown *Hevea* seedlings showed better leaf photosynthetic rate

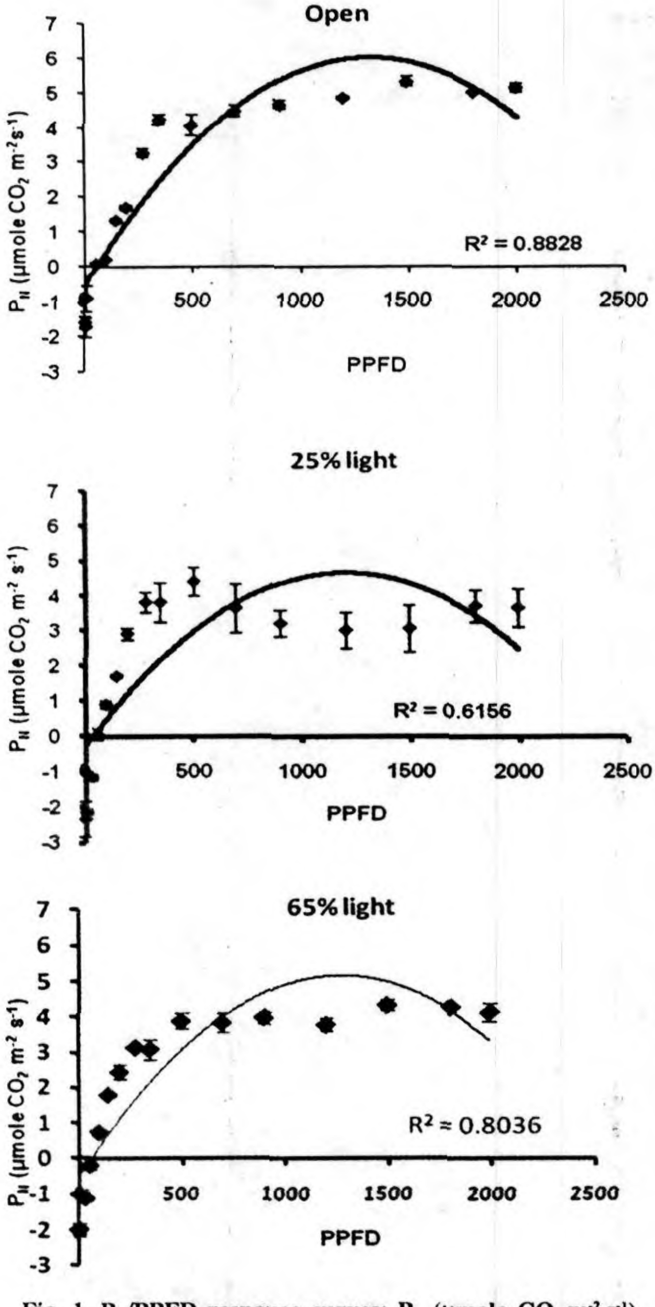

Fig. 1. P_N/PPFD response curves: P_N (µmole CO_2 m^{-2} s^{-1}) affected by different levels of photosynthetic proton flux density (µmole m^{-2} s^{-1}) under three light conditions during experimental period

during the winter period, the above ground biomass attained during this period was higher in partial-shade grown plants. Higher biomass of these seedlings is because of increased average leaf area per plant as compared to full sunlit plants. Shaded plants had more number of leaves per plant than open plants. Early senescence of lower leaves was prominent in full sunlit plants than shaded plants (data not shown). Though higher leaf area per plant was observed in deep-shade grown plants, the growth of these plants was limited by photosynthetic capacity. This reduced photosynthetic capacity was may be due to severe light limitation (Nair *et al.* 2002).

Though partially shaded plants recorded lower photosynthetic rate than open plants, the overall photosynthetic CO_2 assimilation was higher due to larger leaf area and stable photosynthesis even in afternoon hours as well (Alam *et al.* 2005). Under shaded condition the level of photoinhibition was lesser than open light. The production of excess electrons and active oxygen species (AOS) are lower than open plants. Therefore, the shaded plants were protected against lipid and protein peroxidation (Chaves *et al.* 2008). Probably, photorespiration rate is also likely to be less in shaded plants. Therefore, the biomass of plants is gaining steadily when compared to open plants (Moraes *et al.* 2010).

Therefore, a minimal decline in leaf photosynthetic rate under partial shade condition was more than compensated by large increase in leaf area per plant and thus total canopy photosynthesis might be higher in partial-shade grown plants compared to full sunlight grown plants. The plants grown under partial shade condition were taller and also had more dry weight than full sunlight grown plants during low temperature period. Better biomass in *Hevea* seedlings in partially shaded plants may be due to higher net carbon accumulation.

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