PHOTOSYNTHETICA 42 (1): 93-97, 2004

Significance of the leaf area ratio in *Hevea brasiliensis* under high irradiance and low temperature stress

D. RAY*, S.K. DEY, and G. DAS

Regional Research Station, Rubber Research Institute of India,
Rubber Board Kunjaban PO, Agartala, Tripura, 799 006 India

Abstract

Adjustment in leaf area : mass ratio called leaf area ratio (LAR) is one of the strategies to optimize photon harvesting. LAR was recorded for 10 genotypes of *Hevea brasiliensis* under high irradiance and low temperature and the genotypes were categorized into two groups, *i.e.* high LAR and low LAR types. Simultaneously, the growth during summer as well as winter periods, photosynthetic characteristics, and *in-vitro* oxidative damage were studied. Low LAR (19.86±0.52 m^2 kg^{-1}) types, recorded an average of 18.0 % chlorophyll (Chl) degradation under high irradiance and 7.1 % Chl degradation under low temperature. These genotypes maintained significantly higher net photosynthetic rate (P_N) of 10.4 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$ during winter season. On the contrary, the high LAR (24.33±0.27 $m^2\ kg^{-1}$) types recorded significantly lower P_N of 4 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$ and greater Chl degradation of 37.7 and 13.9 % under high irradiance and low temperature stress, respectively. Thus LAR may be one of the physiological traits, which are possibly involved in plant acclimation process under both stresses studied.

Additional key words: CO_2 concentration in sub-stomatal spaces; net photosynthetic rate; *Paraquat*; photo-oxidative damage; stomatal conductance.

Introduction

Plants experience a variety of environmental stresses during their life span. Plant phenotypic acclimation to different combinations of environmental conditions is well-known in most crop species (Boardman 1977, Waller 1986, Le Roux *et al.* 2001). This process of acclimation is very critical for survival and productivity. Leaf phenotypic acclimation, in particular, is crucial for green plants to maintain their metabolic activity under unfavourable irradiance (Le Roux *et al.* 2001). At a given nitrogen availability, leaf photosynthetic activity is acclimated to prevailing irradiance (Field and Mooney 1986, Evans 1989). This phenomenon remains the same even at single leaf level, where leaves can adjust to the incident irradiance (Hirose and Werger 1987, Ackerly 1992, Anten and Werger 1996). Change in leaf mass : area ratio (LAR) is one of the important means, in addition to other different physiological processes, to control photosynthetic acclimation to irradiance in higher plants. Plants optimize the vital processes such as photosynthesis and respiration under high irradiance as the means of acclimation (Marini and Marini 1983, Chazdon and Kaufmann 1993). In several species, photosynthetic acclimation to irradiance is mainly due to adjustment in LAR suggesting that morphological rather than biochemical changes generally govern leaf photosynthetic acclimation to high irradiance (Le Roux *et al.* 1999). Optimization of single leaf area per unit leaf biomass to harvest optimum photon energy is a kind of acclimation to high irradiance in peach (DeJong and Doyle 1985, Rosati *et al.* 1999).

Hevea brasiliensis, when grown in the northeastern region of India, encounters a low temperature stress during winter season (Jacob *et al.* 1999). It defoliates completely during this period and subsequently re-foliates back into full canopy. At that time, leaves experience low temperature of 5–7 °C in early morning followed by high irradiance of 1 500–1 800 $\mu mol\ m^{-2}\ s^{-1}$ during midday. This environmental condition predisposes green leaves to photoinhibitory damage.

To assess the high irradiance acclimation in 10 *Hevea* genotypes, initial characterization was done based on LAR during re-foliation period. On the basis of variation in LAR, six genotypes were classified into two groups. These six selected genotypes were further studied to assess a few attributing traits making them tolerant of high irradiance and low temperature. The gas exchange characteristics and growth pattern were also studied to check genotypic variability in these physiological traits under such stressful environment.

Received 5 June 2003, *accepted* 26 February 2004.

*Author for correspondence; fax: 91-381-2353149, e-mail: deburrii@yahoo.co.in

Materials and methods

Study site and plants: The genotypes of *Hevea brasiliensis* used (MT 5087, MT 4888, RO 4574, MT 5081, MT 4707, MT 4768, AC 4652, MT 4810, and RO 3794) are maintained at Regional Research Farm, Agartala Tripura, NE India (91°15′E, 23°25′N, 30 m a.s.l.). The selection of genotypes was based on the fact that the leaves attained the same physiological maturity at a given time during re-foliation. The soil was sandy loam and all standard cultural practices were followed.

LAR: Leaf samples were taken in triplicates from a composite pool of physiologically mature leaves of 13-year-old trees of each genotype. The leaf area was measured using portable area meter (*LICOR-3000*) and the leaves were oven-dried at 70 °C to constant mass. LAR was calculated as total leaf area per unit leaf dry mass.

Growth of individual plants of each genotype was assessed in terms of the increment in girth of the trunk at 90 cm height. The girth increment was recorded for the periods of January–March (winter) and April–June (summer) using a measuring tape.

Gas exchange studies: Net photosynthetic rate (P_N), stomatal conductance (g_s), and CO_2 concentration in sub-stomatal spaces (C_i) were measured with a portable infrared gas analyzer (*LI 6200*, Lincoln, NE, USA). Measurements were taken on five physiologically mature leaves chosen within the crowns of five trees for each genotype. The gas exchange measurements were taken between 08:00 to 10:00 under saturating irradiance (1 200 μmol $m^{-2} s^{-1}$) and 1.5 kPa of vapour pressure.

***In vitro* studies on photo-oxidative damage**: The photo-oxidative damage was assessed in selected genotypes as the percent chlorophyll (Chl) degradation caused by high irradiance over low irradiance (100 μmol $m^{-2} s^{-1}$), considered as control. *In vitro* application of *Paraquat*, a photosynthetic electron transport-inhibitor, to the leaf discs mimics the oxidative stress under field conditions. The same leaves that were used in gas exchange studies were taken for this experiment. Five leaf discs in ten replications were taken from a composite pool of uniformly mature leaves and incubated in glass plates containing 2 % *Paraquat*. These glass plates were exposed to high irradiance (1 800 μmol $m^{-2} s^{-1}$) for six hours. The leaf discs were spread over wet *Whatman* filter paper to prevent drying of the discs. Another sets of five leaf discs in five replications were kept under 100 μmol $m^{-2} s^{-1}$ irradiance, considered as control. After exposure to high irradiance, Chl amount was estimated in the same leaf discs using the method of Arnon (1949). The percent of Chl degradation was calculated.

Membrane leakage under *in-vitro* low temperature*:* Five uniformly mature twigs, collected from five different trees of each genotype, were brought to the laboratory from the field within half an hour and exposed to 8 °C for 72 h in a *BOD* incubator (*Caltan, DT 909*). An exposure to high irradiance (1 800 μmol $m^{-2} s^{-1}$) followed. The cut ends of the twigs were dipped in water immediately after detaching from the trees. Simultaneously, another set of branches was maintained at room temperature (25 °C) as control. After 72 h, from a composite pool of leaves five leaf discs were sampled in ten replications from both treated and control twigs and incubated in a uniform volume of sterile water in test tubes overnight under a 100 μmol $m^{-2} s^{-1}$ irradiance at 25 °C. Absorbance at 273 nm (A_1) was recorded both in the treated and control samples using the UV spectrophotometer. The same samples were autoclaved at 0.1 mPa pressure for 20 min and the absorbance was recorded again at 273 nm (A_2). The injury to the membrane was calculated using the formula: $[(A_2 - A_1)/A_2]\times100$ (Sullivan and Ross 1979).

The results were analyzed using the *MicroCal Origin* (version *3.0*) *MicrosCal Software* statistical package for *Windows*.

Results and discussion

LAR: All the *H. brasiliensis* genotypes exhibited genetic variability in LAR during re-foliation period. The LAR ranged from 18.05 $m^2 kg^{-1}$ in genotype RO 3794 to 25.14 $m^2 kg^{-1}$ in AC 4652. The genotypes MT 5087, RO 4574, and AC4652 belonged to high LAR group where the low LAR group consisted of RO 3794, MT 4810, and MT 4707 (Table 1). The high LAR types showed an average LAR of 19.86±0.52 $m^2 kg^{-1}$ whereas the other group showed LAR of 24.33±0.27 $m^2 kg^{-1}$.

Growth expressed by girth increment revealed no significant difference between the two groups of plants in summer season. During winter season, low LAR types showed an average girth increment of 0.94 cm, whereas the other category showed only 0.49 cm increment (Table 2).

Gas exchange on uniformly mature leaves of each group of genotypes showed in low and high LAR types average P_N of 10.4 and 4.0 μmol(CO_2) $m^{-2} s^{-1}$, respectively, with g_s of 1.51 and 0.76 mmol $m^{-2} s^{-1}$. An indirect estimate of mesophyll efficiency, the ratio of C_i/g_s, was 257.6 in the low LAR group whereas in the high LAR group it was 446.6 (Table 3). A significant negative relationship was noticed between LAR and P_N (Fig. 1) indicating better photosynthetic efficiency in thicker leaves because of more mesophyll tissue per unit leaf area (Marini and Marini 1983, Reich *et al.* 1991, Dey *et al.* 1995). This may be one of the strategies to acclimate to high irradiance by optimizing photon harvesting.

Table 1. Genetic variability of LAR in *Hevea* genotypes.

Genotype	LAR [$m^2 kg^{-1}$])
RO 3794	18.05
MT 4810	20.71
MT 4707	20.81
AC 4936	21.27
MT 5081	21.73
MT 4768	22.14
MT 4888	22.74
MT 5087	23.51
RO 4574	24.35
AC 4652	25.14
CD (5 %)	9.27

Table 2. Growth of the genotypes during summer (April–June) and winter (January–March) seasons as indicated by girth increment.

	Genotype	Girth increment [cm] Summer	 Winter
Low LAR	MT 4810	0.75±0.12	0.74±0.09
	RO 3794	1.60±0.18	0.90±0.11
	MT 4707	1.00±0.15	1.20±0.13
	Mean	1.11	0.94
High LAR	RO 4574	1.80±0.23	0.81±0.20
	MT 5087	1.00±0.18	0.45±0.11
	AC 4652	0.89±0.16	0.21±0.07
	Mean	1.23	0.49
	CD (5 %)	NS	0.22

Table 3. Gas exchange traits in two groups of *Hevea* genotypes: net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], stomatal conductance, g_s [mmol(CO_2) m^{-2} s^{-1}], and CO_2 concentration in sub-stomatal spaces, C_i [cm^3 m^{-3}].

	Genotype	P_N	g_s	C_i	C_i/g_s
Low LAR	MT 4810	7 44	1.14	366.2	321
	RO 3794	14.27	2.24	360.3	161
	MT 4707	9.49	1.15	335.3	291
	Mean	10.40	1.51	353.9	258
High LAR	RO 4574	3.66	0.84	353.5	420
	MT 5087	4.15	0.72	339.2	470
	AC 4652	4.20	0.73	329.5	450
	Mean	4.00	0.76	340.7	447
	CD (5 %)	1.99	0.58	NS	-

***In vitro* photo-oxidative damage under high irradiance**: Chl degradation due to *in vitro* application of *Paraquat* to the leaf discs under high irradiance revealed in the low LAR genotypes the mean 18.0 % Chl degradation in comparison with 37.7 % in the high LAR genotypes (Fig. 2). *Paraquat* was used in the *in-vitro* experiment to speed up the induction of oxidative stress by blocking the photosynthetic electron transport reactions and thereby producing reactive oxygen species (ROS). Excessive irradiance causes generation of ROS in plants (Sharkey and Vanderveer 1989, Sharkey and Vassey 1989). ROS induce Chl degradation and photosynthetic activity decreases when plants are exposed to irradiance higher than required to saturate photosynthesis (Björkman 1981, Brooks *et al.* 1988).

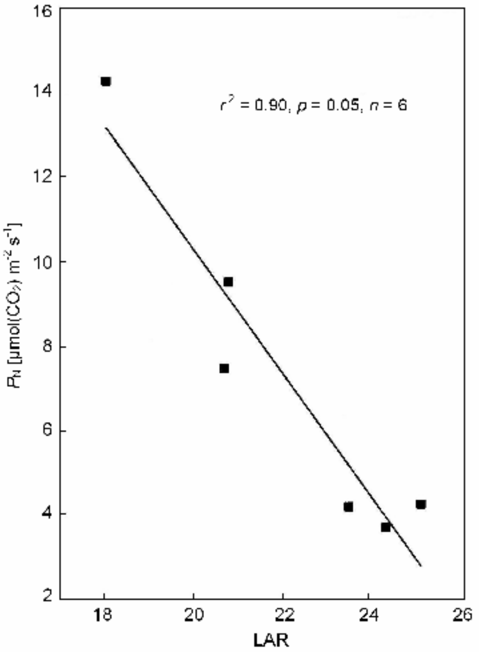

Fig. 1. Relationship between leaf area ratio, LAR and net photosynthetic rate, P_N in six contrasting genotypes of *Hevea*.

Membrane injury caused by low temperature of 8 °C for 72 h was significantly different between the two groups of genotypes. The low temperature ones had the plant tissue predisposed to photoinhibitory membrane injury. The low LAR types experienced lesser membrane injury of 7.1 % whereas in high LAR types it was as high as 13.9 % (Fig. 3). Increased membrane permeability and electrolyte exudate percent was due to membrane injury caused by low temperature (He and China 1986). The structural stability of the plasma membrane is probably maintained in resistant clones whereas it is affected in susceptible clones of *Hevea* (Collins *et al.* 1993, 1995, Sathik *et al.* 1998).

In this study, characterization of a few *Hevea* genotypes revealed the genotypic variability in LAR and photosynthetic traits. The genetic variability in Chl

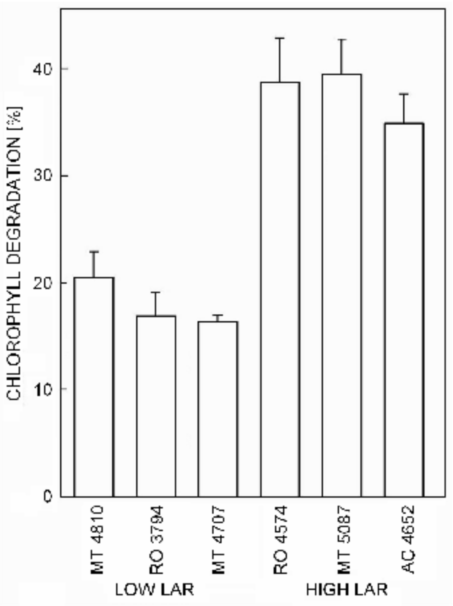

Fig. 2. Variability in chlorophyll degradation caused by *Paraquat in-vitro*: a reflection of oxidative stress in *Hevea* genotypes.

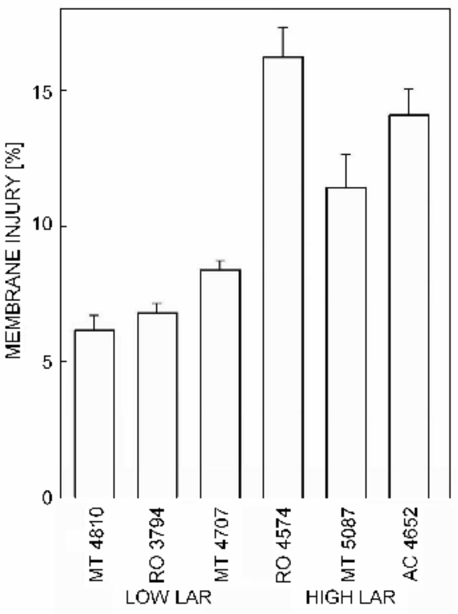

Fig. 3. Genetic variability in membrane injury under low temperature *in vitro* stress in *Hevea* genotypes.

degradation induced by oxidative stress under high irradiance was observed. Similarly, the genotypes varied in membrane injury caused by low temperature. Low LAR or thicker leaves maintained better photosynthetic activity during their re-foliation period. Hence the observed variability in LAR in *H. brasiliensis* germplasm lines may be an adaptation mechanism and is potentially useful in development of thicker leaf genotypes for low temperature and high irradiance tolerance.

References

Ackerly, D.D.: Light, leaf age and leaf nitrogen concentration in a tropical vine. – Oecologia **89**: 596-600, 1992.

Anten, N.P.R., Werger, M.J.A.: Canopy structure and nitrogen distribution in dominant and subordinate plants in a dense stand of *Amaranthus dubius* L. with size hierarchy of individuals. – Oecologia **105**: 30-37, 1996.

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Björkman, O.: Responses to different quantum flux densities. – In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.): Physiological Plant Ecology I. Pp. 57-107. Springer-Verlag, Berlin – Heidelberg – New York 1981.

Boardman, N.K.: Comparative photosynthesis of sun and shade plants. – Annu. Rev. Plant Physiol. **28**: 355-377, 1977.

Brooks, A., Portis, A.R., Jr., Sharkey, T.D.: Effects of irradiance and methyl viologen treatment on ATP, ADP, and activation of ribulose-bisphosphate carboxylase in spinach leaves. – Plant Physiol. **88**: 850-853, 1988.

Chazdon, R.L., Kaufmann, S.: Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. – Funct. Ecol. **7**: 385-394, 1993.

Collins, G.G., Nie, X., Saltveit, M.E.: Heat shock increases chilling tolerance of mung bean hypocotyls tissue. – Physiol. Plant. **89**: 117-124, 1993.

Collins, G.G., Nie, X., Saltveit, M.E.: Heat shock protein and chilling sensitivity of mung bean hypocotyls. – J. exp. Bot. **16**: 795-802, 1995.

DeJong, T.M., Doyle, J.F.: Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (*Prunus persica*). – Plant Cell Environ. **8**: 701-706, 1985.

Dey, S.K., Sobhana, P., Sethuraj, M.R., Vijaykumar, K.R.: Photosynthetic rate and its relation with leaf characteristics in seedlings of *Hevea brasiliensis*. – Indian J. nat. Rubber Res. **8**: 66-69, 1995.

Evans, J.R.: Partitioning of nitrogen between and within leaves under different irradiances. – Aust. J. Plant Physiol. **16**: 533-548, 1989.

Field, C., Mooney, H.A.: The photosynthesis-nitrogen relationship in wild plants. – In: Givnish, G.T. (ed.): On the Economy of Plant Form and Function. Pp. 25-55. Cambridge University Press, Cambridge – London – New York – New Rochelle – Melbourne – Sydney 1986.

He, R., China, Z.: Preliminary studies on cold resistance of rubber trees determined by electric conductometer. – In: Proceeding of the IRRDB Rubber Physiology and Exploitation. Pp. 98-105. SCATC, Hainan 1986.

Hirose, T., Werger, M.J.A.: Maximizing daily canopy photosynthesis with respect to leaf nitrogen allocation pattern in the canopy. – Oecologia **72**: 520-526, 1987.

Jacob, J., Annamalainathan, K., Alam, B., Sathik, M.B., Thapliyal, A.P., Devakumar, A.S.: Physiological constraints for cultivation of *Hevea brasisliensis* in certain unfavorable agro-climatic regions of India. – Indian J. nat. Rubber. Res. **12**: 1-16, 1999.

Le Roux, X., Sinoquet, H., Vandame, M.: Spatial distribution of leaf dry weight per area and leaf nitrogen concentration in relation to local radiation regime. – Tree Physiol. **19**: 181-188, 1999.

Le Roux, X., Walcroft, A.S., Daudet, F.A., Sinoquet, H., Chaves, M.M., Rodrigues, A., Osorio, L.: Photosynthetic light acclimation in peach leaves: importance of changes in mass:area ratio, nitrogen concentration, and leaf nitrogen partitioning. – Tree Physiol. **21**: 377-386, 2001.

Marini, R.P., Marini, M.C.: Seasonal changes in specific leaf weight, net photosynthesis and chlorophyll content of leaves of peach leaves as affected by light penetration and canopy position. – J. amer. Soc. hort. Sci. **108**: 609-613, 1983.

Reich, P.B., Uhl, C., Walters, M.B., Ellsworth, D.A.: Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species. – Oecologia **86**: 16-24, 1991.

Rosati, A., Esparza, G., DeJong, T.M., Pearcy, R.W.: Influence of canopy light environment and nitrogen availability on leaf photosynthetic characteristics and photosynthetic nitrogen use efficiency of field-grown nectarine trees. – Tree Physiol. **19**: 173-180, 1999.

Sathik, M.B.M., Vijayakumar, K.R., Jacob, J., Sethuraj, M.R.: Membrane stability as measured by electrolyte leakage: A tool for screening *Hevea* clones for cold tolerance. – In: Mathew, N.M., Jacob, K. (ed.): Developments in Plantation Crop Research. Pp. 136-138. Rubber Research Institute of India, Kottayam 1998.

Sharkey, T.D., Vanderveer, P.J.: Stromal phosphate concentration is low during feedback limited photosynthesis. – Plant Physiol. **91**: 679-684, 1989.

Sharkey, T.D., Vassey, T.L.: Low oxygen inhibition of photosynthesis is caused by inhibition of starch synthesis. – Plant Physiol. **90**: 385-387, 1989.

Sullivan, C.Y., Ross, W.M.: Selecting for drought and heat resistance in grain sorghum. – In: Mussel, H., Staples, R.C. (ed.): Stress Physiology in Crop Plants. Pp. 264-281. John Wiley, New York 1979.

Waller, D.M.: The dynamics of growth and form. – In: Crawles, M.J. (ed.): Plant Ecology. P. 496. Oxford University Press, Oxford – London 1986.