Low night temperature-induced changes in photosynthesis and rubber accumulation in guayule (*Parthenium argentatum* Gray)

D. SUNDAR and A. RAMACHANDRA REDDY*

School of Life Sciences, Pondicherry University, Pondicherry 605 014, India

Abstract

Three-year-old plants of *Parthenium argentatum* Gray cv. 11591 grown under natural photoperiod were exposed for 60 d to low night temperature (LNT) of 15 °C (daily from 18:00 to 06:00). Effects of the treatment on net photosynthetic rates (P_N), rubber accumulation, and associated biochemical traits were examined. LNT initially reduced P_N with a parallel decline in the activities of ribulose-1,5-bisphosphate carboxylase, fructose bisphosphatase, and sucrose phosphate synthase for 20-30 d. Later, LNT enhanced P_N and the activities of photosynthetic enzymes. Associated with high P_N in LNT-treated guayule plants was a two-fold increase in rubber content and rubber transferase activity per unit of protein. The initial decrease in P_N in LNT-treated guayule was associated with low content of chlorophyll ($a+b$), large starch accumulation, and higher ratio of glucose-6-phosphate/fructose-6-phosphate. Photosystem 2 activity in isolated chloroplasts was initially decreased, but increased after 30 d. There was a significant increase in the leaf soluble protein content in LNT-treated plants. Hence the photosynthetic performance of plants grown at 15 °C night temperature for 50 d was superior to those grown under natural photoperiod in all parameters studied. The high photosynthetic capacity may contribute to superior rubber yields under LNT.

Additional key words: chlorophyll; fructose-6-phosphate; glucose-6-phosphate; net photosynthetic rate; photosystems 1 and 2; proteins; rubber transferase; starch; saccharose; sucrose phosphate synthase; superoxide dismutase.

Introduction

Low temperature is a major factor limiting the geographical locations suitable for crop growth and periodically accounts for significant losses in plant production. Short term exposure of plants to low temperature usually inhibits P_N due to accumulation of soluble saccharides and reduced orthophosphate cycling from the cytosol back to the chloroplast and therefore limits ATP synthesis needed for RuBP regeneration (Ebrahim *et al.* 1998, Hurry *et al.* 1998). Irradiation of barley and cucumber leaves exposed to chilling temperature results in photoinhibition of photosystem 1 (Tjus *et al.* 1999). Long-term exposure of evergreen woody perennials such as Scots pine to low growth temperature results in a depression of radiant energy-saturated P_N (Öquist and Martin 1986). In contrast, long term acclimation (as in *Nerium oleander* and *Eucalyptus* sp.) and net growth and development at low temperature, results not only in the accumulation of soluble saccharides, but also in an increase in P_N (Ferrar *et al.* 1989, Holaday *et al.* 1992). The photosynthetic capacity of herbaceous annuals recovers following growth at low temperature through increases in the activities of several photosynthetic enzymes (Hurry *et al.* 1995). During moderate chilling of cotton, enhanced activities of enzymes related to starch and other soluble saccharides were observed (Perera *et al.* 1995). In addition to its effects on P_N, low temperature also results in the expression of dehydrins that help to protect the plant from dehydration (Bravo *et al.* 1999). Thus the existing literature provides evidence for significant positive and negative changes associated with primary metabolism in several plants after low growth temperature treatment. However, the effects of low temperature on secondary metabolic pathways and the accumulation of various secondary metabolites in higher plants are poorly understood.

Received 28 August 2000, *accepted* 23 October 2000.
*Author for correspondence; fax: +91-413-655211/655265; e-mail: arreddy@yahoo.com
Abbreviations: Chl, chlorophyll; DM, dry mass; DTT, dithiothreitol; F6P, fructose-6-phosphate; FBPase, fructose bisphosphatase; FPP, farnesyl pyrophophate; G6P, glucose-6-phosphate; GSH, reduced glutathione; IPP, isopentenyl pyrophosphate; LNT, low night temperature; NTB, nitroblue tetrazolium; PS, photosystem; PVP, polyvinylpyrrolidone; RuBP, ribulose-1,5-bisphosphate; RuBPC, ribulose-1,5-bisphosphate carboxylase; SPS, sucrose phosphate synthase; UDPG, uridine-5-diphosphoglucose.
Acknowledgements: This work was funded by grant BT/R&D/08/018/95 from the Department of Biotechnology, Govt. of India to A. Ramachandra Reddy. A Junior Research Fellowship to D. Sundar from this project is gratefully acknowledged.

About 2 500 plant species produce polymeric natural rubber (*cis*-1,4-polyisoprene) from isoprenoid monomers (C_5H_8) derived from isopentenyl pyrophosphate (Backhaus 1998). All natural rubber currently used is obtained from a single tropical species, *Hevea brasiliensis* Muell. Arg. Nonetheless, a woody shrub guayule (*Parthenium argentatum* Gray) is currently being exploited as a domestic source of natural rubber. One major factor delaying the economical commercialisation of guayule is its low rubber yield (Estilai and Waines 1987). Hence the primary emphasis on the guayule breeding programmes has been to increase the rubber content and quality. Cultivation of guayule in arid climates is under active development. Unlike the laticiferous species, guayule produces rubber in the parenchyma cells of root and stem. Rubber formation in guayule is cyclic (Bonner 1943). Most rubber is produced during the cool temperatures of fall and winter as the plant is exposed to LNT which may increase the expression of genes coding for enzymes involved in rubber synthesis (Bonner 1975). Although low temperature-mediated rubber formation in guayule was due to increased activity of rubber transferase (Goss *et al.* 1984), the temperature dependence of the overall basic metabolic processes in guayule is not well understood. We have previously reported the role of photosynthetic and photorespiratory metabolites in providing precursors for rubber biosynthesis (Ramachandra Reddy and Das 1987, Ramachandra Reddy *et al.* 1987). The main objective of the present study was to characterise the changes in the photosynthetic carbon metabolism, rubber formation, and accumulation patterns in guayule under LNT treatments.

Materials and methods

Biochemicals: Radiochemical (1-^{14}C) isopentenyl pyrophosphate (IPP, specific activity 2035 GBq mol^{-1}) was obtained from *Amersham Pharmacia Biotech International* (UK) and $NaH^{14}CO_3$ (specific activity 1942.5 GBq mol^{-1}) was obtained from Bhabha Atomic Research Center, Mumbai, India. All other biochemicals were purchased from *Sigma* (St Louis, MO, USA).

Plants of guayule (*Parthenium argentatum* Gray) cv. 11591 were grown in 30-cm pots under natural photoperiod. Plants received full solar irradiance for most of the day in a 12 h photoperiod. The maximum irradiance (PAR, 400-700 nm) available at the top of the canopy was 1800 to 2000 μmol m^{-2} s^{-1} on a clear day. Daily maximum and minimum air temperatures were 29-33 °C and 20-22 °C, respectively. The plants were watered regularly and fertilised weekly with nutrient solution of [mM] 14 N, 6 P, and 12 K. The plants were subjected to LNT treatment of 15 °C by transferring the pots to the walk-in growth chamber (*Lab Line*, 104 A, IL, USA). The LNT treatment was given for 12 h daily (each night from 18:00 to 06:00) for 60 nights. During daytime, the plants were grown outside under natural photoperiod as described above.

P_N was measured according to Ramachandra Reddy and Nandan Kumar (1996). Photosynthetic rates were monitored after pre-irradiation of the leaves for 40 min. Irradiation was by halide flood lamps behind a water screen. P_N measurement for individual leaves was repeated three to four times on different individual shoots.

Rubber content: The stem portions of control and LNT-treated guayule were cut into pieces and dried in an oven at 64 °C until constant mass was obtained. The dried tissue was ground in a mill through 40-mesh screen, the powder was thoroughly mixed, and samples were placed in cellulose extraction thimbles (*Whatman*) and extracted with acetone for 16 h in a Soxhlet apparatus to remove resins. The extraction thimbles were then dried and further extracted for 16 h in the Soxhlet apparatus. Hexane extracts containing the dissolved rubber were made up to 25 cm^3 in a volumetric flask. A 2-cm^3 aliquot was taken into a cuvette and 6 cm^3 of acidified ethanol was added as a precipitating agent. The cuvettes were agitated and allowed to stand for 20 min. The per cent transmittances of the samples were measured at 750 nm (Naqvi *et al.* 1984). Transmittances were compared with a standard curve prepared with pure natural rubber.

Isolation of chloroplasts and estimation of photosystem activities: Freshly harvested leaves were irradiated for 15 min. The leaves were cut into strips and homogenised in a semi-frozen grinding medium which consisted of 0.33 M sorbitol, 10 mM $Na_4P_2O_7$, 5 mM $MgCl_2$, 1 % polyvinyl pyrrolidone, 0.5 mM dithiothreitol (DTT), and 2 mM sodium arsenate. The crude extract was squeezed through two layers of cheesecloth and the filtrate centrifuged at 250×*g* for 5 min to remove cell debris. The supernatant was then centrifuged at 2 500×*g* for 10 min. The pellet was suspended in a cold medium consisting of 0.33 M sorbitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 50 mM HEPES (pH 7.6). A portion of this chloroplast preparation was layered on to a sucrose gradient comprising 1.5, 1.0, and 0.75 M sucrose in 10 mM Tricine-KOH (pH 7.6) and centrifuged at 2 500×*g* for 15 min. The chloroplasts at the interface between 1.0 and 1.5 M sucrose were diluted with a suspension medium consisting of 0.33 M sorbitol, 50 mM HEPES (pH 7.6), 2 mM EDTA, 1 mM $MgCl_2$, and 1 mM $MnCl_2$. This suspension was centrifuged at 5000×*g* for 5 min to yield a pellet of intact purified chloroplasts. The intactness of the purified chloroplasts used in the present

study was 80 to 85 % according to Lilley *et al.* (1975). The photochemical activities in isolated chloroplasts were determined spectrophotometrically as described by Raghavendra and Das (1976).

Enzyme activities: All extractions were performed at 4 °C. The leaf blades (10 g) were homogenised with 50 cm^3 of 100 mM Tris-HCl buffer (pH 7.8) containing 5 mM DTT, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM magnesium acetate, and 1.5 % PVP-40. The homogenate was squeezed through four layers of cheesecloth and then centrifuged at 10 000×*g* for 10 min. The solution was filtered off to remove the cellulose and washed thrice with the extraction medium. The protein was precipitated with 75 % (m/v) ammonium sulphate and spun at 30 000×*g* for 30 min. The precipitate was dissolved in 50 mM Tris-HCl buffer (pH 7.8) containing 1 mM DTT and 2 mM EDTA. The preparation was applied to a column of *Sephadex G-25*, equilibrated with 100 mM Tris-HCl (pH 8.0) which contained 1 mM DTT, 10 mM $NaHCO_3$, 20 mM $MgCl_2$, and 0.2 mM NaDPH. The eluates were collected at room temperature.

RuBPC activities were assayed at 30 °C by the incorporation of $^{14}CO_2$ into acid stable products (Lorimer *et al.* 1977). The assay mixture contained (3 cm^3): 100 mM Tris-HCl buffer (pH 8.0), 5 mM DTT, 20 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$ (7.4 GBq mol^{-1}), 0.5 mM RuBP, and the enzyme extract. Reaction was started by the addition of 20 mm^3 of the activated enzyme and stopped after 60 s by adding 0.3 cm^3 of 2 M acetic acid. The radioactivity was measured in a *Beckman LS 1800* liquid scintillation counter. Fructose-1,6-bisphosphatase was assayed according to Zimmerman *et al.* (1978). The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM DTT, 1 mM EDTA, 0.5 mM NADP, 1 mM fructose-1,6-bisphosphate, 10 units each of glucose phosphate isomerase and glucose-6-phosphate dehydrogenase, and the enzyme extract. Sucrose phosphate synthase was assayed at 30 °C by measuring the production of UDP (Huber 1981). The reaction mixture (2.0 cm^3) contained 50 mM HEPES-NaOH (pH 7.5), 8 mM UDPG, 8 mM fructose-6-P, 10 mM $MgCl_2$, and the enzyme. By adding 0.1 cm^3 of 1 M NaOH the reaction was terminated. The tubes were kept in boiling water bath for 10 min to remove the non-reacted F6P. After cooling, 0.5 cm^3 of 0.1 % (v/v) resorcinol in 95 % ethyl alcohol and 1.0 cm^3 of 30 % HCl were added. The tubes were incubated at 80 °C for 10 min. The reaction tubes were cooled, and the absorbance at 520 nm was recorded. Superoxide dismutase activity was determined spectrophotometrically at 560 nm according to Dhindsa and Matowe (1981). The reaction mixture (1 cm^3) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM nitroblue tetrazolium (NTB), 2 μM riboflavin, and 100 mm^3 of the enzyme extract. Chlorophyll (Chl) content in the leaf extracts was determined according to Arnon (1949).

Estimation of chloroplast metabolites: Starch and sucrose contents in the leaf tissues were estimated according to Ramachandra Reddy *et al.* (1996). For starch content, eight leaf discs (1-cm diameter) were selected at random from the leaves and extracted 4 to 5 times with 80 % ethanol for 15 min at 80 °C. The extracts were combined and stored for estimating sucrose. The ethanol-extracted leaf discs were suspended in 1 cm^3 of 0.2 M KOH and boiled for 0.5 h. The tubes were cooled to room temperature, 0.2 cm^3 of 1 M acetic acid was added to each tube, and reacted for 0.5 h at 55 °C to hydrolyse starch. The reaction was stopped by treating for 60 s at 100 °C. The contents were cooled and brought to a known volume (6 cm^3). A 0.2-cm^3 aliquot of the extract was added to 0.3 cm^3 of distilled water and 1 cm^3 of glucose-enzyme reagent (*Sigma 115*). The tubes were incubated at 37 °C for 20 min and the absorbance was read at 492 nm. For the estimation of sucrose content, the ethanol extracts, previously described, were used. The extract (0.2 cm^3) was added to 0.3 cm^3 of glucose reagent and incubated for 0.3 h at 37 °C. Invertase (75 mm^3, *Sigma I 4753*) was added and incubated for 0.5 h at 37 °C. The absorbance was read at 492 nm. This reading is proportional to the original plus glucose liberated *via* invertase action on sucrose. To determine the sucrose content, the assays were run simultaneously (lacking invertase) for glucose content. Sucrose concentration was determined by comparing the difference in the absorbance of the two samples with that obtained from the sucrose standards. The contents of G6P and F6P were estimated as described by Leegood (1993).

Extraction and assay of rubber transferase: Samples of stem portions were collected from guayule plants at each experimental condition and separately extracted for the enzyme. The samples were rinsed with distilled water and homogenised in a pre-cooled Waring blendor with 100 mM Tris-HCl buffer (pH 7.5) consisting of 2 mM $MnSO_4$ and 0.1 mM GSH. The homogenate was filtered through eight layers of cheesecloth. Then it was centrifuged at 30 000×*g* for 45 min at 2 °C. The supernatant was fractionated with solid ammonium sulphate. The protein that precipitated between 40 and 60 % saturation was collected by centrifuging at 25 000×*g*. The protein was dissolved in a small volume of extraction buffer and desalted by passing through a column of *Sephadex G-25* pre-equilibrated with the extraction buffer. The fractions were pooled and assayed for rubber transferase.

Rubber transferase was assayed according to Cornish and Backhaus (1990) with certain modifications. The reaction mixture (1 cm^3) contained 50 mM Tris-HCl buffer (pH 7.5), 0.3 mM $MgSO_4$, 3 mM GSH, 5 mM DTT, 25 μM FPP, 86.5 μM 1-^{14}C-IPP (40.7 GBq mol^{-1}),

and the enzyme protein (100 μg). Incubations for 1 h at 30 °C were terminated by the addition of 0.5 cm^3 of 0.2 M EDTA. The contents in the reaction tubes were dried in a stream of air at 70 °C. The rubber films were saponified and coagulated as described by Madhavan and Benedict (1984). The rubber coagulate was dissolved in 2 cm^3 of 1 % trichloroacetic acid in toluene, scintillant was added to give a total volume of 6 cm^3, and the solutions were counted for radioactivity in a scintillation counter. Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin as the standard protein.

Results

Under LNT, the guayule leaves showed a decline in P_N in the initial stages and later a gradual increase (Fig. 1*A*). Plant exposed to 60 nights of LNT exhibited higher P_N (1.00 mg m^{-2} s^{-1}) than the control plants (0.86 mg m^{-2} s^{-1}).

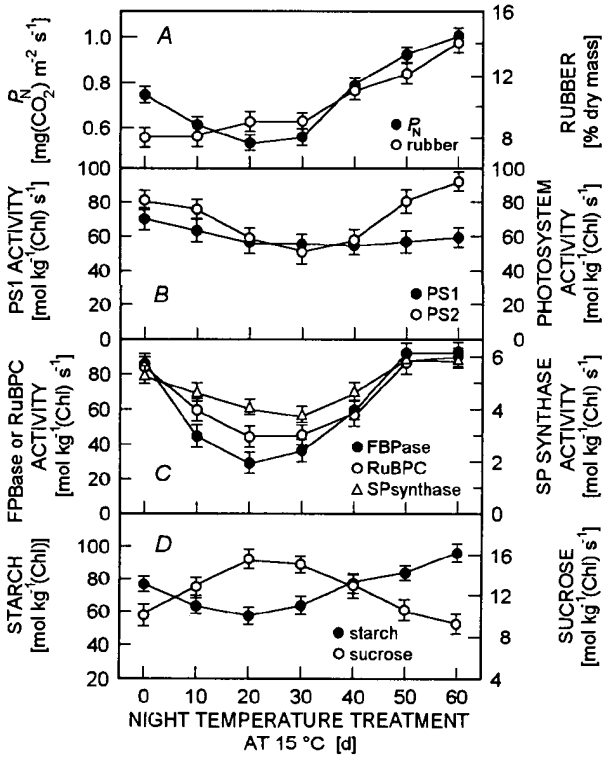

Fig. 1. Effect of low night temperature (15 °C) treatment on net photosynthetic rate P_N, rubber accumulation (*A*), and photosystem 2 and 1 activities in isolated and purified chloroplasts (*B*), activities of RuBPC, fructose bisphosphatase (FBPase), and sucrose phosphate synthase (SPsynthase) (*C*), and the contents of starch and sucrose in guayule leaves. Each point is an average of at least four replications ± SE.

Guayule plants exposed to LNT also possessed significantly high rubber content after 60 nights (14 % DM) compared to that in control plants (8 % DM, Fig. 1*A*). LNT treatment caused an initial decrease in the activity of photosystem (PS) 2 and later increase while the PS1 activity in isolated chloroplasts was unaffected by LNT treatment (Fig. 1*B*). Marked changes in the activities of key photosynthetic enzymes were noticed in leaf extracts of LNT plants (Fig. 1*C*). The activities of RuBPC, fructose-1,6-bisphosphatase, and sucrose phosphate synthase declined up to 20 d of LNT treatment and later rapidly increased up to 50 d of treatment. The

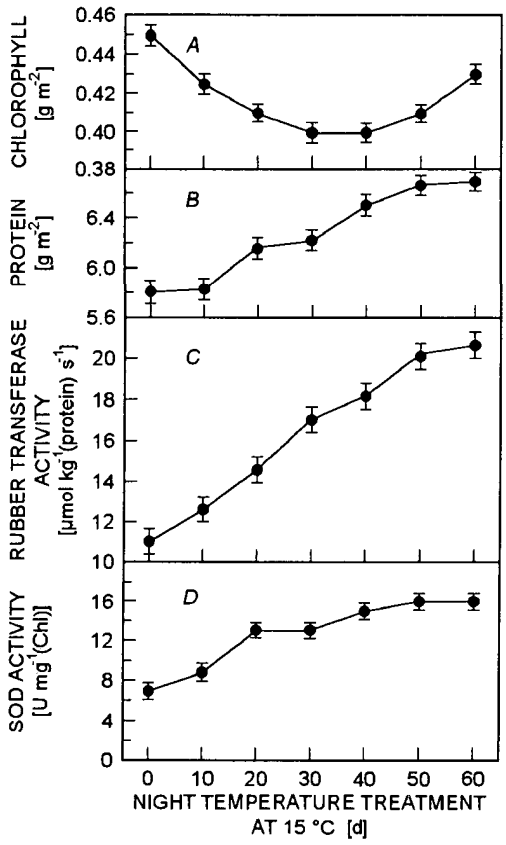

Fig. 2. Effect of low night temperature (15 °C) treatment on chlorophyll (*a*+*b*) content (*A*), soluble protein content (*B*), activity of rubber transferase (*C*), and the activity of superoxide dismutase (*D*) in guayule leaves. Each point is an average of at least four replications ± SE.

activities of these three enzymes during the LNT treatment were positively correlated with leaf P_N (Fig. 1*A,C*). LNT initially caused a substantial increase in the foliar starch content in guayule while the content of sucrose showed an initial decline followed by progressive increase from 20 d of treatment (Fig. 1*D*). Similarly the ratios of G6P/F6P were closely associated with the

Table 1. Foliar contents of glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) [mol kg^{-1}(Chl)] in guayule as affected by low night temperature treatment at 15 °C (LNT). The metabolites were assayed in the leaf extracts at 11:00. Each value is an average of at least four replications.

Days of LNT treatment	G6P	F6P	G6P/F6P
0	201	115	1.75
10	262	105	2.49
20	305	80	2.49
30	301	72	4.18
40	255	92	2.77
50	206	130	1.58
60	179	132	1.36

activity of sucrose phosphate synthase and sucrose formation during LNT treatment (Table 1). LNT treatment reduced Chl content up to 40 d but later the leaves accumulated more Chl (Fig. 2*A*). Conversely, total leaf soluble protein content showed a progressive increase with increased time of LNT treatment (Fig. 2*B*). LNT significantly enhanced the activity of rubber transferase in guayule stem extracts. The enzyme extracts from 60-d-treated guayule plants showed approximately two-fold increase in the activity which were well correlated with the rubber accumulation under LNT treatment (Fig. 1*A*, 2*C*). The extractable activity of superoxide dismutase measured in leaves on Chl basis increased significantly with LNT treatment (Fig. 2*D*).

Discussion

The exposure of plants to low temperature induces many changes in physiological and biochemical parameters. Several studies investigated the relationship between low temperature treatment and enzyme activities (Thomashow 1999). Guayule produces more rubber during winter and LNT favours more rubber formation (Madhavan *et al.* 1989). However, the mechanism of stimulation of rubber formation in guayule by LNT is still elusive. Photosynthetic metabolites serve as good precursors for certain secondary metabolites including rubber in higher plants (Ramachandra Reddy and Das 1987, 1995, Heintze *et al.* 1994). However, the relationship of photosynthetic capacity in guayule and rubber formation has not been documented. In this study, we provide an evidence that photosynthetic carbon assimilation is crucial for rubber formation in guayule.

Rubber and similar metabolites use acetyl Co-A as the initial substrate and ATP and NADPH as cofactors (Benedict 1983, Ramachandra Reddy and Das 1987). The availability of these three metabolites would provide a mechanism for regulating the secondary metabolite production superimposed upon controls at the enzyme and cellular level. Acetyl CoA, ATP, and NADPH are ubiquitous cellular metabolites derived either from stroma saccharides or arising directly from photosynthesis (Heintze *et al.* 1994, Ramachandra Reddy and Das 1995). We thus expect a close correlation between P_N and production of rubber in guayule. In a wide range of plant species, experimental manipulations that enhanced P_N enhanced the pace of secondary metabolite production (Gershenzon and Croteau 1993). Photosynthetically fixed carbon is actively incorporated into isoprenoids (Goodwin 1965, Heintze *et al.* 1994). Although it was presumed that the precursor is produced in the leaf and is translocated to the stem and root for polymerisation (Benedict 1983, Gilliland *et al.* 1985), there exists some ambiguity about the precursor used for rubber production in guayule. Therefore, the role of photosynthetically fixed carbon and the availability of photosynthates is very significant for regulating rubber formation and accumulation in guayule. We found that LNT treatment resulted in a relative increase in P_N after an apparent acclimation of about 20 d. P_N in LNT-treated plants was strongly correlated with the accumulation of rubber. LNT strongly affected the photosynthate content as indicated by changes in the ratio of G6P/F6P. After acclimation of 20 d to LNT, the photosynthetic enzyme activities were significantly high in leaf extracts indicating the increased photosynthetic capacity in guayule leaves. Increased contents of sucrose and other saccharides reflect the increased capacity for photosynthesis in guayule that might lead to increased rubber biosynthesis utilising the photosynthetic intermediates as potential precursors. The regulation and activities of fructose bisphosphatase and sucrose phosphate synthase are more important in determining the carbon flux through the path of sucrose synthesis in guayule. However, increased starch accumulation in the initial stages of LNT treatment would have resulted in lower activities of PS2 and associated reduction in photosynthesis. We also presume that increased activities of sucrose phosphate synthase after acclimation could trigger more sucrose synthesis, which might provide more carbon skeleton for rubber formation under such favourable conditions.

The initial decline in P_N after LNT treatment might be due to low fructose bisphosphatase and sucrose phosphate synthase activities and also to changes in photochemistry of chloroplasts associated with high starch content. Our results, for the first time, show a positive correlation between increased P_N and rubber formation in guayule upon LNT treatment. We favour the view that good correlation results from photosynthesis providing more precursors necessary for rubber formation in guayule. The changes in photosynthetic activity in guayule leaves at LNT were correlated well with the activities of

RuBPC, fructose bisphosphatase, and sucrose phosphate synthase implying that LNT increased their activation states. We presume that increased sucrose synthesis after acclimation to LNT treatment reflects the increased photosynthetic capacity. P_N in guayule acclimated to LNT for 60 d was much higher than in control plants indicating that guayule plants acclimated to LNT have high intrinsic capacity for sucrose synthesis. In this study, LNT-treated guayule leaves showed more than twice as much activity of superoxide dismutase as in control plants that evidence that guayule leaves have a potential antioxidant system to protect against low temperature-induced oxidative damage (Doulis *et al.* 1997). The ability of guayule to acclimate the key photosynthetic enzymes and to raise P_N with LNT treatment is a significant feature that could contribute to low temperature resistance which in turn also increases rubber yields under LNT. The adaptive feature of guayule in facing LNT indicates the greater potential of this crop for superior photosynthetic performance at temperatures far off from optimum. In addition, this study provides an evidence for the implications of LNT treatment for carbon gain required for rubber biosynthesis and also illustrates how a single physiological trait such as photosynthesis might have functional consequences in different habitats of guayule.

References

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

Backhaus, R.A. : Natural rubber from plants. – In: Kaplan, D.L. (ed.): Biopolymers from Renewable Resources. Pp. 323-354. Springer-Verlag, Berlin 1998.

Benedict, C.R.: Biosynthesis or rubber. - In: Porter, J.W., Spurgeon, S.L. (ed.): Biosynthesis of Isoprenoid Compounds. Pp. 357-369. John Wiley & Sons, New York 1983.

Bonner, J.: Effects of temperature on rubber accumulation in the guayule plant. – Bot. Gaz. **105**: 233-243, 1943.

Bonner, J.: Physiology and chemistry of guayule. – In: McGinnies, N.G., Haase, E.F. (ed.): An International Conference on the Utilization of Guayule. P. 78. Office of Land Studies, University of Arizona, Tucson 1975.

Bradford, M.A.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. – Anal. Biochem. **72**: 248-254, 1976.

Bravo, L.A., Close, T.J., Corcuera, L.J., Guy, C.L.: Characterization of a 80-kDa dehydrin-like protein in barley responsive to cold acclimation. – Physiol. Plant. **106**: 177-183, 1999.

Cornish, K., Backhaus, R.A.: Rubber transferase activity in rubber particles of guayule. – Phytochemistry **29**: 3809-3813, 1990.

Dhindsa, R.S., Matowe, W.: Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. – J. exp. Bot. **32**: 79-91, 1981.

Doulis, A.G., Debian, N., Kingston-Smith, A.H., Foyer, C.H.: Differential localisation of antioxidants in maize leaves. - Plant Physiol. **114**: 1031-1037, 1997.

Ebrahim, M.K.H., Vogg, G., Osman, M.E.H., Komor, E.: Photosynthetic performance and adaptation of sugarcane at suboptimal temperatures. - J. Plant Physiol. **153**: 587-592, 1998.

Estilai, A., Waines, J.G.: Variation in regrowth and its implications for multiple harvest of guayule. - Crop Sci. **27**: 100-103, 1987.

Ferrar, P.J., Slatyer, R.O., Vranjic, J.A.: Photosynthetic temperature acclimation in *Eucalyptus* species from diverse habitats and a comparison with *Nerium oleander*. – Aust. J. Plant Physiol. **16**: 199-217, 1989.

Gershenzon, J., Croteau, R.B.: Terpenoid biosynthesis: the basic pathway and formation of monoteprenes, sesquiterpenes and diterpenes. – In: Towers, G.H.N., Stafford, H.A. (ed.): Lipid Metabolism in Plants. Pp. 339-388. CRC Press, Boca Raton 1993.

Gilliland, M.G., Van Staden, J., Mitchell, J.J.: CO_2 fixation and rubber deposition in the bark and leaves of guayule during winter. – J. Plant Physiol. **121**: 369-379, 1985.

Goodwin, T.W.: Regulation of terpenoid synthesis in higher plants. – In: Pridham, J.B., Swain, T. (ed.). Biosynthetic Mechanisms in Higher Plants. Pp. 57-71. Academic Press, London 1965.

Goss, R.A., Benedict, C.R., Keithly, J.H., Nessler, C.L., Stipanovic, R.D.: *Cis*-polyisoprene synthesis in guayule plants (*Parthenium argentatum* Gray) exposed to low, non-freezing temperatures. - Plant Physiol. **74**: 534-537, 1984.

Heintze, A., Riedel, A., Aydogdu, S., Schultz, G.: Formation of chloroplast isoprenoids from pyruvate and acetate by chloroplasts from young spinach plants. Evidence for a mevalonate pathway in immature chloroplasts. - Plant Physiol. Biochem. **32**: 791-797, 1994.

Holaday, A.S., Martindale, W., Alred, R., Brooks, A.L., Leegood, R.C.: Changes in activities of enzymes of carbon metabolism in leaves during exposure of plants to low temperature. - Plant Physiol. **98**: 1105-1114, 1992.

Huber, S.C.: Interspecific variation in activity and regulation of leaf sucrose phosphate synthetase. – Z. Pflanzenphysiol. **102**: 443-450, 1981.

Hurry, V., Huner, N., Selstam, E., Gardestrom, P., Oquist, G.: Photosynthesis at low growth temperatures. – In: Raghavendra, A.S. (ed.): Photosynthesis. A Comprehensive Treatise. Pp. 238-249. Cambridge University Press, Cambridge 1998.

Hurry, V.M., Strand, A., Tobiaeson, M., Gardestrom, P., Oquist, G.: Cold hardening of spring and winter wheat and rape results in differential effects on growth, carbon metabolism and carbohydrate content. - Plant Physiol. **109**: 697-706, 1995.

Leegood, R.C.: Carbon metabolism. – In: Hall, D.O., Scurlock, J.M.O., Bolhàr-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.): Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual. Pp. 247-267. Chapman and Hall, London – Glasgow – New York – Tokyo – Melbourne – Madras 1993.

Lilley, R.McC., Fitzgerald, M.P., Rienits, K.G., Walker, D.A.: Criteria of intactness and the photosynthetic activity of

spinach chloroplast preparations. - New Phytol. **75**: 1-10, 1975.

Lorimer, G.H., Badger, M.R., Andrews, T.J.: D-ribulose-1,5-bisphosphate carboxylase-oxygenase. Improved methods for the activation and assay of catalytic activities. – Anal. Biochem. **78**: 66-75, 1977.

Madhavan, S., Benedict, C.R.: Isopentenyl pyrophosphate cis-1,4-polyisoprenyl transferase from guayule (*Parthenium argentatum* Gray). - Plant Physiol. **75**: 908-913, 1984.

Madhavan, S., Greenblatt, G.A., Foster, M.A., Benedict, C.R.: Stimulation of isopentenyl pyrophosphate incorporation into polyisoprene in extracts from guayule plants (*Parthenium argentatum* Gray) by low temperature and 2-(3,4-dichlorophenoxy) triethylamine. - Plant Physiol. **89**: 506-511, 1989.

Naqvi, H.H., Younger, V.B., Rodriguez, E.: Inheritance of rubber content and morphological traits in F_1 hybrids between *Parthenium argentatum* (guayule) and *Parthenium schottii*. – Bull. Torrey bot. Club **111**: 377-382, 1984.

Öquist, G., Martin, B.: Cold climates. – In: Baker, N.R., Long, S.P. (ed.): Photosynthesis in Contrasting Environments. Pp. 237-293. Elsevier Science Publ., Amsterdam 1986.

Perera, N.H., Hartmann, E., Holaday, A.S.: Regulation of cotton photosynthesis during moderate chilling. - Plant Sci. **111**: 133-143, 1995.

Raghavendra, A.S., Das, V.S.R.: Distribution of C_4 dicarboxylic acid pathway of photosynthesis in local monocotyledonous plants and its taxonomic significance. - New Phytol. **76**: 301-305, 1976.

Ramachandra Reddy, A., Das, V.S.R.: Chloroplast autonomy for the biosynthesis of isopentenyl diphosphate in guayule (*Parthenium argentatum* Gray). - New Phytol. **106**: 457-464, 1987.

Ramachandra Reddy, A., Das, V.S.R.: Utilization of photosynthates during rubber biosynthesis in guayule (*Parthenium argentatum* Gray). - Photosynthetica **31**: 351-358, 1995.

Ramachandra Reddy, A., Nandan Kumar, S.: Enhanced photosynthesis in cytozyme-treated blackgram, *Phaseolus mungo* L. – Ind. J. exp. Biol. **34**: 702-705, 1996.

Ramachandra Reddy, A., Reddy, K.R., Hodges, H.F.: Mepiquat chloride (PIX)-induced changes in photosynthesis and growth of cotton. - Plant Growth Reg. **20**, 179-183, 1996.

Ramachandra Reddy, A., Suhasini, M., Das, V.S.R.: Impairment of photorespiratory carbon flow into rubber by the inhibition of the glycolate pathway in guayule (*Parthenium argentatum* Gray). - Plant Physiol. **84**: 1447-1450, 1987.

Thomashow, M.F.: Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. – Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 571-599, 1999.

Tjus, S.E., Møller, B.M., Scheller, H.V.: Photoinhibition of Photosystem I damages both reaction centre proteins PSI-A and PSI-B and acceptor-side located small Photosystem I polypeptides. – Photosynth. Res. **60**: 75-86, 1999.

Zimmerman, G., Kelly, G.J., Latzko, E.: Purification and properties of spinach leaf cytoplasmic fructose-1,6-bisphosphatase. – J. biol. Chem. **253**: 5952-5956, 1978.