ATP concentration in latex as an indicator for early evaluation of yield in *Hevea brasiliensis*

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Abstract

Adenosine 5' triphosphate (ATP) in the laticiferous tissues of *Hevea brasiliensis* plays a major role in rubber biosynthesis through its direct involvement in metabolic pathways and indirectly through H^+ ATPase activity. A strong positive correlation was observed between dry rubber yield and latex ATP, when ATP was measured in the latex of ten *Hevea* clones with different yield potentials such as low, medium and high. The latex ATP was significantly high in clones with higher yield than medium and low yielding clones. The correlation between same day latex yield and ATP during the peak yielding season (September-November) also showed a direct relationship. This study was extended to immature plants of the same clones to confirm the practical application of this finding in crop improvement programme. Latex ATP was analysed in two year old young plants and continued for five years to correlate with rubber yield of the mature trees in field trial. A positive correlation was noticed between ATP in young plants and mature tree rubber yield. Young plants of high yielding clones always showed higher latex ATP concentration. In view of the direct relationship with yield, significant differences between clones and its seasonal insensitivity, latex ATP could be used as an indicator for early prediction of high yield in *Hevea*.

Keywords: ATP, energy metabolism, *Hevea brasiliensis,* latex production

Introduction

Energy metabolism plays an important role in latex production in *Hevea brasiliensis*. Harvesting latex from rubber tree on a regular basis causes intense metabolic activity in laticifers to regenerate rubber and other subcellular components, mainly proteins and nucleic acids during subsequent tapping. A large supply of energy is thus required to sustain these processes. ATP (Adenosine-5'-triphosphate) and ADP (Adenosine-5'-diphosphate) are important physiological regulators in the whole metabolic pathway leading to rubber biosynthesis and latex production. The quantity and the rate of turnover of adenylate pools (ATP, ADP and AMP) have fundamental importance in the regeneration and flow of latex and consequently on rubber yield (Jacob *et al.*, 1997).

Besides releasing energy which is used in various metabolic pathways, ATP is also the specific substrate for the H^+ pumping ATPase located on the lutoid membrane (d'Auzac, 1977; Chretin, 1982). This H^+ ATPase regulates the pH of the cytosol by pumping protons from the cytosol to the lutoid resulting in enhanced catabolism of sugars and activating several pH dependent enzymes. ATP also provides energy for the transport of various solutes in and out of the lutoids both of which influence latex production (Marin *et al.*, 1981).

The pool sizes of adenylates, adenylate energy charge (AEC), lutoid membrane ATPase and C-serum pH in different clones with high and low yield were studied during peak yielding and stress season (Sreelatha *et al.*, 2004). There was a strong positive correlation among concentration of ATP in latex, lutoid membrane ATPase activity and

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cytosolic pH and all these three parameters were significantly higher in high yielding clones than the low yielding clones during peak yielding season. Concentration of ATP in the latex remained relatively high in high yielding clones and also positively correlated with latex yield during the summer season also.

Considering the regulatory role of ATP in rubber biosynthesis and its seasonal insensitivity, earlier workers had suggested the possibility of using latex ATP as an indicator of high yield in *Hevea brasiliensis* (Sreelatha *et al.*, 2004). In the present study, more number of clones with different yield potentials at mature stage and the young plants of the same clones were selected to establish the potential of using ATP as a marker for early evaluation of yield and to make use of it in the crop improvement programme.

Materials and methods

Plant material- Mature trees

The study was carried out in ten *Hevea* clones *viz.*, RRII 105, RRIM 600, PB235, PB260, PB 217 (clones representing high yielders); GT1, PB 5/51, Tjir1 (clones representing medium yielders) and RRII 38, RRII 33 (clones representing low yielders). The planting was done during 1992 at the research farm of Rubber Research Institute of India, Kottayam, Kerala. The clones were categorized based on the yield data collected for five years after opening the trees for tapping. The trees selected in this study were in the eighth year of tapping under S/2 d3 6d/7 system. Nine mature trees from each clone with uniform yield and girth were selected. Yield and ATP were measured during the peak yielding season (September-November) and the data were analysed statistically. For regression analysis total latex volume (ml tree^{-1} tap$^{-1)}$ and same day latex ATP content were used.

Immature plants

Polybag plants of the same ten clones were planted at Central Experiment Station, Chethackal during 2005 in randomized block design with fifty plants per clone to study the relation between latex ATP content in young plants and mature tree rubber yield. Fifteen trees per clone were selected for measurement of latex ATP. Latex samples were collected from young plants by pricking the trees with needle for the initial three years; fourth and fifth year latex samples were collected by making small tapping cuts using a sharp knife. The data collected during peak yielding season (September-November) were pooled together to study the correlation between ATP content and rubber yield during immature stage and mature stage of *Hevea*.

Estimation of ATP

ATP concentration in latex was determined according to Amalou *et al.* (1992) with some modifications. Fresh latex samples were extracted with 2.5 per cent trichloroacetic acid (TCA). An aliquot was neutralized with 0.1N KOH and made up to a known volume with 30mM HEPES (N-[2-Hydroxy ethyl] piperazine-N'-[2-ethane sulphonic acid])-Tris (hydroxymethyl aminomethane) buffer pH 7.4 and quantified using bioluminescent kit (FL-AA-Sigma Chemical Company, USA). The data collected were analysed statistically. ANOVA and DMRT were carried out to determine the mean differences in dry rubber yield and latex ATP between clones. Correlation was worked out with dry rubber yield (g tree^{-1} tap^{-1}) and latex ATP as well as with ATP content and latex yield (ml tree^{-1}tap^{-1}) in the corresponding days.

Results and discussion

Mature yield and ATP

The data on dry rubber yield of ten *Hevea* clones in the first five years after tapping and the mean yield over five years were presented in Table 1. Significantly high dry rubber yield in clones RRII 105, RRIM 600, PB 217, PB 235 and PB 260 compared to medium (PB 5/51 and Tjir 1) and low yielding clones (RRII 38 and RRII 33) were noticed. GT 1, a clone showing rising yield trend, recorded better mean yield in this trial.

During the peak yielding season, all the high yielding clones (RRII 105, PB 235 and PB 260) except PB 217 showed significantly high latex ATP and the low yielding clones (RRII 33 and RRII 38) showed very low ATP content in latex. The clone GT 1, classified as medium yielder showed high latex ATP similar to high yielders. The other two medium yielders Tjir1 and PB 5/51 showed intermediate ATP concentration (Table 2).

Table 1. Rubber yield (g $t^{-1}t^{-1}$) of *Hevea* clones in the first five years of tapping

Clones	Yield					Mean yield
	1st year	2nd year	3rd year	4th year	5th year	over five years
RRII 105	34.6 bc	58.40 b	68.3 b	84.6 a	74.9 b	64.1 b
RRIM 600	29.43 cd	40.73 d	48.4 cd	55.5 c	57.4 c	46.3 c
PB 217	35.27 bc	47.17 cd	51.3 cd	54.3 c	48.5 d	47.3 c
PB 235	40.47 b	69.00 a	78.0 a	84.5 a	87.4 a	70.9 a
PB 260	48.6 a	53.83 bc	77.2 a	69.6 b	82.4 a	66.3 ab
PB 5/51	21.17 ef	26.37 e	26.0 e	33.4 d	39.3 e	29.6 e
Tjir 1	18.93 f	30.77 e	43.0 d	46.3 c	43.3 de	36.4 d
GT1	26.97 de	47.63 cd	54.1 c	49.9 c	49.5 d	45.2 c
RRII 38	8.47 g	11.03 f	11.3 f	13.4 f	16.7 f	11.7 f
RRII 33	8.3 g	8.57 f	20.4 e	23.0 e	16.4 f	15.8 f

(Mean values followed by common alphabets are not significantly different at P ≤ 05)

Table 2. Rubber yield during peak season (Sep-Nov) and latex ATP in different *Hevea* clones

Clones	Yield (g $t^{-1}t^{-1}$)	ATP (µM)
RRII 105	91.8a	278.12a
RRIM 600	61.5b	253.67bc
PB 217	55.0bc	218.45de
PB 235	63.2b	232.96cd
PB 260	59.9b	245.05bc
PB 5/51	36.3cd	197.66e
Tjir 1	35.7cd	202.61e
GT1	46.9bc	263.26ab
RRII 38	25.7d	145.13f
RRII 33	15.6d	144.60f

(Mean values followed by common alphabet are not significantly different at P ≤ 0.05)

All the data collected during peak yielding season were pooled together and a correlation was established between latex ATP and dry rubber yield. A highly significant positive correlation between latex ATP and rubber yield was observed among different clones (r = 0.69). The regression analysis of latex yield with ATP also showed a direct relationship (Figs. 1-3) during the peak yielding months (September, October and November). This result suggests that latex ATP has a direct role in controlling rubber biosynthesis. A large supply of ATP can make rubber biosynthesis more efficient not only by providing more energy for the conversion of sucrose to polyisoprene but also by raising the cytosolic pH through ATPase activity (Sreelatha *et al.*, 2004). Thus, ATP is a very important regulator in the rubber biosynthetic pathway through its direct involvement by supplying energy and indirectly through ATPase activity. This fact was further supported by

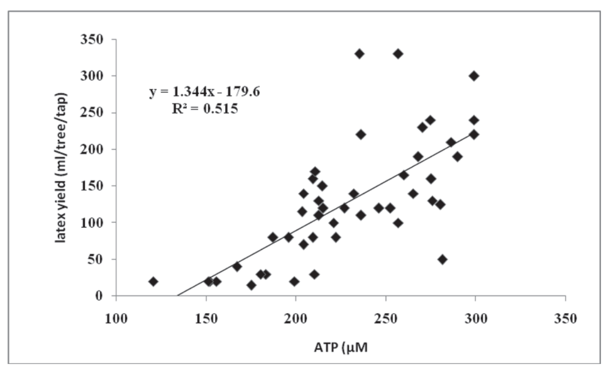

Fig. 1. Relationship between latex yield and ATP during September

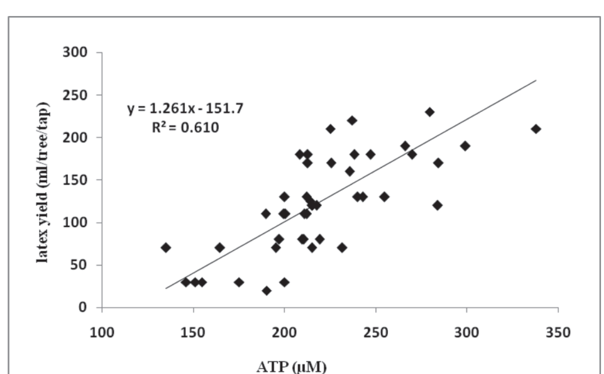

Fig. 2. Relationship between latex yield and ATP during October

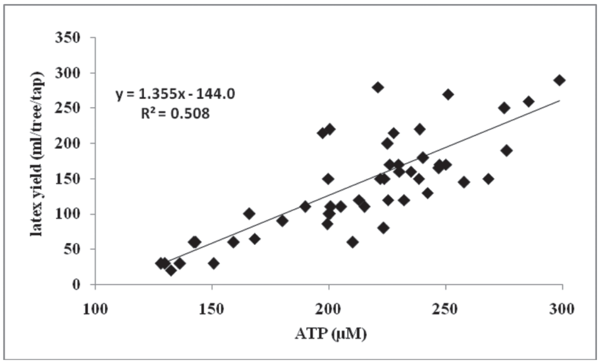

Fig. 3. Relationship between latex yield and ATP during November

observation in tapping panel dryness affected (TPD) trees where inhibition of rubber biosynthesis was associated with significantly lower concentrations of ATP in the latex (Krishnakumar *et al.*, 2001). The high yielding clones RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430 showed higher concentrations of ATP in their latex and the variations in latex ATP during peak yielding and stress season in these clones and the interaction with yield was also studied (Sreelatha *et al.*, 2009; Sreelatha *et al.*, 2011). Moreover lutoid membrane H^+ ATPase activity is always controlled by the availability of ATP in the latex and its real activity depends on cytosol ATP content (Chrestin and Gidrol, 1986; Chretin, 1982). Early activation of tonoplast H^+ pumping ATPase by ethylene was also reported by Gidrol *et al.*, (1988). Stimulation of regularly tapped trees induced a variation in latex ATP content two hour after the treatment. In stimulated trees, after a transient decrease, the ATP content increased rapidly and exceeding the levels of unstimulated control (Amalou *et al.*, 1992).

Immature yield and ATP

This study was further extended to immature plants of the same clones to evaluate the potential of ATP in early determination of yield in *Hevea* for crop improvement programme, The same ten clones were planted in the field and latex ATP was analysed from second year of planting onwards and correlated with the yield data of the corresponding clones in the mature trial. Young plants of high yielding clones showed high latex ATP compared to low yielding clones (Table 3). On the contrary, the clone PB 217 which is a high yielder showed a lower ATP value as indicated in the mature phase. This clone has been reported to have high sucrose content with a slow metabolic rate (Prevot *et al.*, 1986). The medium yielding clone GT1 always showed higher ATP content as seen in high yielding clones (Table 3). Low yielding clones always recorded very low latex ATP levels. Correlation between latex ATP at immature phase and dry rubber yield at mature phase of the corresponding clones is presented in Table 4. Significant positive correlation was observed on all the years and the correlation became more stable when the plants are attaining maturity.

In *Hevea*, several parameters were tested for early evaluation of yield in the nursery plants

Table 3. Latex ATP (μM) in immature plants of ten *Hevea* clones of different yield potential

Clones	Yield class	2nd year	3rd year	4th year	5th year
RRII 105	High	339.28^{a}	334.84^{a}	294.76^{a}	297.53^{a}
RRIM 600	High	306.21^{ab}	304.25^{ab}	236.04^{bcd}	239.85^{b}
PB 217	High	253.7^{bc}	245.77^{bc}	204.25^{cd}	207.91^{c}
PB 235	High	306.67^{ab}	286.61^{ab}	257.19^{ab}	239.68^{b}
PB 260	High	284.55^{ab}	282.85^{ab}	222.38^{bcd}	228.42^{bc}
PB 5/51	Medium	197.72^{cd}	208.28^{cd}	194.96^{d}	199.54^{c}
Tjir 1	Medium	277.99^{ab}	244.47^{bc}	204.16^{cd}	207.09^{c}
GT1	Medium	319.96^{a}	304.65^{ab}	247.96^{bc}	246.75^{b}
RRII 38	Low	192.32^{d}	174.80^{d}	157.08^{e}	136.19^{d}
RRII 33	Low	190.03^{d}	155.02^{d}	148.02^{e}	142.19^{d}

(Mean values followed by common alphabet are not significantly different at $P \leq 0.05$)

Table 4. Correlation between mature yield over five years of different clones and latex ATP in immature plants

	Latex ATP			
	2 year old plants	3 year old plants	4 year old plants	5 year old plants
Mature tree yield over five years	0.706**	0.523**	0.78**	0.78**

** Correlation is significant at the 0.01 level (2-tailed)

(Ho *et al.*, 1973; Chandrasekhar and Gireesh, 2009; Mydin, 2012) and these parameters could help to reduce the population by eliminating the poor yielding plants at the nursery stage itself. The correlation between rubber yield and various yield influencing physiological parameters which were directly or indirectly related to latex yield and their interrelationship were studied by several workers (Eschbach *et al.*, 1984; Prevot *et al.*, 1986; Wititsuvannakul *et al.,* 1988; Bricard and Nicolas. 1985; Sreelatha, 2003; Simon, 2003). The relative importance and reliability of any physiological marker depend on the association of marker with yield at both immature and mature stages. Given the great importance of ATP in rubber biosynthesis and consistently high degree of positive correlation observed with dry rubber yield at immature and mature phase suggests the potential of latex ATP as a biochemical marker for yield at the juvenile stage in *Hevea brasiliensis*. Measurement of ATP is simple, reliable and stable method which can be done using a small volume of latex. This parameter would strengthen the early prediction of yield potential at juvenile stage and can be employed in the crop improvement programme.

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