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ORIGINAL ARTICLE

Role of lutoid membrane transport and protein synthesis in the regeneration mechanism of latex in different *Hevea* clones

S. Sreelatha[1] · Sheela P. Simon[1] · V. C. Mercykutty[1] · Kavitha K. Mydin[1] · R. Krishnakumar[1] · K. Annamalainathan[1] · James Jacob[1]

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Abstract Natural rubber (cis-1,4 polyisoprene) is synthesised in the milky cytoplasm, the latex, of specialized cells called laticifers in the bark tissues of the rubber tree (*Hevea brasiliensis*). Regeneration mechanism of latex after each tapping (controlled wounding of the bark) was studied in relation to lutoid membrane enzymes and protein synthesis in twelve rubber clones with varying yield potentials during the peak rubber yielding season. High activity of membrane enzymes and better availability of biochemical energy [ATP] were observed in clones viz; RRII 105, RRIM 600, PB 260, RRII 422 and RRII 430. The highest protein biosynthetic capacity was noticed in clone PB 260 and RRIM 600. However, high ATP content, increased invertase activity and protein biosynthesis were observed in the medium yielding clone GT1 compared to clones with low rubber yield potential. Very low sugar content and increased invertase activity in the latex of clone PB 260 indicated intense latex metabolism with high protein turnover that implies fast recouping of the cellular metabolites lost during latex harvesting. Clone PB 217 was characterized by very high sucrose and low ATP concentration and ATPase activity in latex indicating slow metabolism and hence be suitable for inducing latex metabolism using ethylene stimulant. Low rubber yielding clones such as RRII 33 and RRII 38 were consistently recorded a high sucrose content but very low activity of membrane enzymes, reduced ATP concentration and low protein biosynthesis in latex. Among the recently released modern clones (RRII 400 series), latex regeneration capacity was higher in RRII 422 and RRII 430. The significance of lutoid membrane transport and protein synthesis is discussed in relation to general latex metabolism of these rubber clones. The outcome of this study would be helpful to design suitable latex harvesting systems and yield stimulation methods for optimizing latex production in each clone based on metabolic profiling.

Keywords *Hevea brasiliensis* · Lutoids · Latex regeneration · Membrane transport · Metabolism

Introduction

Hevea brasiliensis (Rubber Tree) is an economically important crop cultivated for the production of Natural Rubber (long chain *cis*-1,4 polyisoprene). Rubber is synthesized and stored in specialized cells called laticifers in phloem. Rubber particles and small single membrane bound vacuolar structures (lutoids) constitute large portion of latex (D'Auzac et al. 1982). The latex is collected from the trees by making controlled wounding of the bark of the tree called tapping. While tapping, the pure cytoplasmic contents of the laticiferous vessels expelled in the form of latex. Latex flow after tapping and regeneration of cellular materials before the next tapping are the two main factors that limit rubber production in *Hevea brasiliensis* (Jacob et al. 1985). Latex flow depends on turgor pressure; water transport and coagulation mechanism by bursting of the lutoid bodies. Latex regeneration is controlled by different biochemical and molecular mechanisms such as supply of sucrose (unique precursor for rubber synthesis) to laticifers, availability of biochemical energy [ATP], proton transport

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✉ S. Sreelatha
sreelatha@rubberboard.org.in

[1] Rubber Research Institute of India, Kottayam 686009, Kerala, India

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mechanism in the lutoid membrane, pH regulation and also the capacity of laticifers to regenerate the cellular metabolites lost during tapping (Chrestin and Gidrol 1986; Jacob et al. 1997; Tang et al. 2013).

Lutoids which comprises nearly 20 % of the latex volume and are involved in stopping of the latex flow gradually after a period of 1–2 h after tapping (D'Auzac et al. 1982). Lutoids accumulate various components such as Pi, citrate, Mg, proteins, basic amino acids and acid hydrolases. The release of these materials into the latex leads to coagulation of latex and stopping the flow of latex after a period of tapping. Lutoid membrane enzymes viz H^+ ATPase and H^+ pyrophosphatase (which are energized by ATP and PPi) are involved in the transport of protons from the cytosol to lutoids (Siswanto et al. 1994; Jacob et al. 1997; Marin et al. 1981). The efficient pumping of protons from cytosol to lutoids resulted in the activation of carbohydrate metabolism by activating several pH dependent enzymes mainly invertase. The synthesis and turnover of proteins is also very important in the overall metabolic rate during rubber synthesis. All these factors are very essential for the sustainable latex production.

Latex production can be optimized by manipulating the tapping frequency and stimulation of latex yield by applying ethylene compounds such as ethephon, a commercial grade of ethylene compound (2-chloro ethyl phosphonic acid) in the bark of the tree. Treatment with ethephon results in prolonged latex flow and is one of the main reasons for increased latex yield through exogenous application of this compound (Abraham et al. 1968; D'Auzac et al. 1997). Carbohydrate metabolism, availability of ATP, protein biosynthesis and several metabolic enzymes has been shown to be regulated by ethylene in rubber tree (Gidrol et al. 1988; Coupe and Chrestin 1989; Amalou et al. 1992). Increased sucrose transport into laticifers after stimulation (Dusotoit-Coucaud et al. 2009) and molecular mechanism for regulation of latex regeneration and duration of flow at transcriptome level (Chao et al. 2015) were also reported. The objective of the present experiment is to evaluate the biochemical parameters and correlate with the latex regeneration mechanism of different *Hevea* clones with varying yield potentials.

Materials and methods

Plant material

The study was carried out in two different trials, Trial-1 with seven *Hevea* clones (RRII 105, PB 260, RRIM 600, PB 217, GT1, RRII 33 and RRII 38) planted during 1992 at the Rubber Research Institute of India (RRII), Kottayam, Kerala, India (76°36′E and 9°32′N) and Trial 2 with five newly released RRII 400 series clones (RRII 414, RRII 417, RRII 422, RRII 429, RRII 430) along with the popular high yielding control clone RRII 105 planted during 1993 at Central Experiment Station of the Rubber Research Institute of India, Chethackal, Kerala, India (76°5′E and 9°22′N). The rubber tree clone RRII 105 is a hybrid of Tjir 1 and Gl 1. Clones PB 260 (hybrid of PB 5/51 × PB 49), RRIM 600 (hybrid of Tjir 1 × PB 86), PB 217 (hybrid of PB 5/51 × PB 6/9) are introduced from Malaysia. Clone GT1 (primary clone evolved by ortet selection) is introduced from Indonesia. RRII 33 and RRII 38 are ortet selections of India. RRII 400 series clones were evolved by hybridization of RRII 105 × RRIC 100 followed by clonal selection. The design of the experiment was complete randomized block replicated three times with a plot size of 16 trees each. Nine trees (three trees from each replication) having comparable girth and latex yield were selected from each clone and the trees of both the trials were tapped regularly. The tapping system followed in this experimental trees was S/2 d3 6d/7 (half spiral cut under third daily frequency, 6 days in tapping followed by 1-day tapping rest. The tapping notation followed is as reported by Vijayakumar et al. (2009). Latex samples were collected from the trees during the peak crop yielding season (August–November) for two consecutive years and measured the biochemical parameters. This period is considered ideal for biochemical measurements as latex is more stable during this time.

Latex collection and preparation of cytosol and lutoids

The first 5 ml latex flowing immediately after tapping was discarded and the subsequent latex was collected in vials in an ice bath. The cold latex samples were then centrifuged at 4 °C for 45 min (59,000×*g*, Sorvall OTD 55B Ultra centrifuge, T865 rotor). The top cream consisting of rubber particles were discarded and the middle aqueous layer was cytosol (C-serum) which was collected using syringe. The bottom fraction consisting of the vacuolar compartment (lutoids) were collected and washed in 0.4 M mannitol and suspended in five volumes of 50 mM HEPES (N-[2-hydroxy ethyl] piperazine-N′-[2-ethane sulphonic acid])-MES- (2-[N-morpholino] ethane sulphonic acid)- Tris buffer pH 7.0. The crude lutoid fraction was washed three times with same buffer and centrifuged at 35,000 g for 30 min at 4 °C. The bottom pellet was collected as purified lutoids (Gidrol et al. 1988).

Isolation of lutoid membrane and protein extraction

The purified lutoids were suspended in five volumes of washing buffer [20 mM Tris–HCl, 300 mM mannitol, 0.5 mM Dithiothreitol (DTT) pH 7.2] and submitted to

repeated freezing (−20 °C) and thawing (room temperature) to rupture the lutoid membrane and releasing the fluid content. The membrane was collected by centrifuging the mixture at 39,000×*g* for 30 min and resuspended in membrane protein extraction buffer and centrifuged at 51,000×*g* for 30 min. Protein content were measured according to Lowry et al. (1951) using the standard protein (Bovine Serum Albumin).

Rubber yield determination

Annual rubber yield of each clone was determined after recording yield of individual trees at fortnightly intervals by cup coagulation method. After stopping the latex flow, 3 % formic acid was added into the collection cup and stirred to accelerate latex coagulation. The coagulated rubber (cup lump) of individual trees was collected, dried in smoke house and then weighed to constant weight and expressed as g of dry rubber per tree per tapping after correcting the moisture content (Nair et al. 2012).

The rubber yield of experimental trees during the peak yielding season was calculated from the total latex volume obtained after each tapping and dry rubber content (drc) of the latex (rubber yield = total volume of latex × drc/100). The rubber yield was expressed as g/tree/tap. The drc (%) of latex was determined by coagulating 5 g latex using 2 % acetic acid. The rubber coagulum was washed and pressed to thin sheet and dried at 60 °C and weighed until constant weight is obtained and calculated as dry weight of latex/fresh weight of latex × 100.

Determination of ATP

The ATP concentration in latex was determined according to Amalou et al. (1992) with certain modifications. ATP was extracted from fresh latex samples by treating with 2.5 % trichloroacetic acid (TCA). The coagulated rubber was rinsed twice with TCA and made up to a known volume and filtered. An aliquot was neutralized with 0.1 N KOH and made up to a known volume with 30 mM HEPES (N-[2-hydroxy ethyl] piperazine-N′-[2-ethane sulphonic acid])-Tris (hydroxymethyl aminomethane) buffer pH 7.4 and quantified using ATP bioluminescent kit (FL-AA-Sigma Chemical Company, USA) which contain luciferin-luciferase enzyme and pure ATP standard. A luminometer (Stratech) was used for the measurement. ATP concentration of the whole latex was expressed as μM.

Estimation of sucrose

Latex (1 g) was extracted using 2.5 % TCA and made up to a known volume, filtered and an aliquot was used for estimating sucrose content following the procedure of Scott and Melvin (1953) and expressed as mM.

Assay of invertase (EC 3.2.1.26) in C-serum (cytosol)

Clear cytosol (C-serum) after centrifugation of latex was used for the determination of invertase following the method of Tupy (1973) with certain modifications. C-serum (50 μl) was incubated in a reaction medium contained 2 ml 50 mM phosphate buffer (pH 7.2), 0.06 mM sucrose and 0.01 mM sodium fluoride at 30 °C for 1 h and stopped the reaction by adding 2 volumes of absolute alcohol and by boiling for 5 min. The reaction mixture was centrifuged and the supernatant was used for measuring the glucose content. The activity of invertase was expressed as nmol glucose liberated/min/mg protein.

Assay of lutoid membrane ATPase (EC 3.6.3.6)

Activity of H^+ ATPase was measured in purified lutoid membrane according to Gidrol et al. (1988) with modifications. Assay was performed in 2.5 ml buffer (50 mM Hepes-Mes-Tris pH 7.0, 300 mM mannitol, 5 mM $MgSO_4$, 0.1 mM ammonium molybdate with 20 μg/ml lutoid membrane protein. The reaction was started by addition of 100 μl 5 mM ATP. The incubation time was 10 min at 26 °C under continuous stirring. The reaction was stopped by adding 200 μl ice cold 0.5 mM TCA. The inorganic phosphorous released during the hydrolysis of ATP by ATPase was measured spectrophotometrically (Taussky and Shorr 1953) and expressed as μM Pi liberated/min/mg protein.

Assay of lutoid membrane pyrophosphatase (EC 3.6.1.1)

Activity of H^+ pyrophosphatase was measured in purified lutoid membrane according to Siswanto et al. (1994) with some modifications. The reaction medium contained 30 mM Tris-MES pH 8.0, 1 mM MgSO4, 0.5 mM sodium fluoride, 50 mM potassium chloride, 1 mM sodium pyrophosphate and 30 μg/ml lutoid membrane protein. The released inorganic phosphorous was measured spectrophotometrically (Taussky and Shorr 1953) and expressed as μM Pi liberated/min/mg protein.

In vitro protein biosynthesis

In vitro protein synthesis assay (Gidrol and Chrestin 1984) was carried out in C-serum using a mixture of ^{14}C labeled amino acids (arginine, leucine, lysine and phenyl alanine). The cytosolic serum was incubated in a medium containing 10 mM mannitol, 12 mM fructose, 15 mM reduced

glutathione, 3 mM cysteine, 6 mM ATP, 6 mM GTP, 1.5 mM NAD, 0.3 mM ammonium molybdate, 1 mM chloramphenicol, a mixture of 19 amino acids containing 2 mg each and 0.25 μCi/ml of ^{14}C labeled arginine, leucine, lysine and phenyl alanine. The samples were incubated for 4 h at room temperature and stopped the reaction by adding 1 ml of cold (unlabeled) amino acid mixture (100 mM arginine, leucine, lysine and phenyl alanine) and 1 mg/ml cycloheximide. Proteins were precipitated with 5 % TCA and washed three times with 5 % TCA, 50 mM KCl and 10 mM of each aminoacid. The precipitated protein was dissolved in 0.5 N NaOH, kept overnight and measured the radioactivity using a scintillation counter (LKB).

Statistical analysis

Analysis of variance and the Duncan's multiple range test (DMRT) were employed for comparing the mean values of biochemical parameters between clones.

Results and discussion

Rubber yield of different clones

The mean dry rubber yield over 5 years after opening the trees for harvesting latex of the clones in Trial-1 are summarized in Table 1. Based on the rubber yield, the clones were categorized as high, medium and low yielding clones. Analysis of variance of the data showed a significantly high dry rubber yield in clones viz; RRII 105 and PB 260 compared to the yield of other clones studied. RRIM 600, PB 217 and GT1 are medium yielding clones. Mean rubber yield over a period of 5 years consistently showed very low in clones RRII 33 and RRII 38 (less than 20 g/tree/tap). During the peak rubber yielding period also (September–November), clones RRII 105 and PB 260 showed significantly high dry rubber yield (Table 2) than the medium yielding clones (RRIM 600, PB 217 and GT1) and low yielding clones (RRII 33 and RRII 38).

Superior growth and yield potential of RRII 400 series clones compared to RRII 105 were well established in small scale and large scale trials across the traditional rubber growing regions of India (Mydin and Mercykutty 2007). Rubber yield of the modern RRII 400 series *Hevea* clones were reported by Mydin et al. (2011). RRII 422 and RRII 430 had higher dry rubber yield (68.57 g/tree/tap and 72.3 g/tree/tap) over 8 years of tapping (Mydin et al. 2011) than the control clone RRII 105 (57.4 g/tree/tap). The experimental trees of RRII 400 series clones (Trial-2) were selected from the same trial. During the experimental period (peak rubber yielding season- August–November), RRII 422 and RRII 430 showed significantly high dry rubber yield ($p < 0.05$, Table 3) than RRII 105. Yield of other RRII 400 series clones (RRII 414, RRII 417 and RRII 429) were comparable to that of RRII 105 clone. Since the two trials were conducted at two different locations, the popular high yielding clone RRII 105 was selected as a control clone for both the experiments for comparison of biochemical data. Clone characterization was made using yield and biochemical data collected during the peak yielding season in both the trials.

Latex sucrose, ATP and invertase in different clones

Out of the seven clones studied in Trial-1, highest sucrose content was observed in clone PB 217 (above 25 mM) and the lowest in clone PB 260 (2.6 mM). RRIM 600 (medium yielding clone) and the low yielding clones such as RRII 38 and RRII 33 showed same sucrose level in latex. Sucrose level of clone GT1 is comparable to that of RRII 105. RRII 105 had higher ATP and invertase activity in latex compared to medium and low yielding clones. GT1 also showed significantly high ATP and invertase activity than that of low yielding clones. The low yielding clones RRII 33 and RRII 38 had very low ATP and invertase in latex compared to high and medium yielding clones (Table 2).

Table 1 Rubber yield (g/tree/tap) of different *Hevea* clones in the first 5 years after tapping and mean yield over 5 years (Trial-1)

Clones	Yield					Mean yield over 5 years
	1st year	2nd year	3rd year	4th year	5th year	
RRII 105	34.6^{bc}	58.4^{a}	68.3^{b}	84.6^{a}	74.9^{b}	64.1^{a}
PB 260	48.6^{a}	53.6^{ab}	77.2^{a}	69.6^{b}	82.4^{a}	66.3^{a}
RRIM 600	29.4^{bc}	40.7^{c}	48.4^{d}	55.5^{c}	57.5^{c}	46.3^{b}
PB 217	35.2^{bc}	47.1^{bc}	51.3^{cd}	58.3^{c}	51.8^{cd}	48.7^{b}
GT1	26.9^{c}	47.6^{abc}	54.2^{c}	50.9^{c}	49.5^{d}	45.8^{b}
RRII 33	8.3^{d}	11.0^{d}	20.4^{e}	23.0^{d}	16.4^{e}	15.8^{c}
RRII 38	8.4^{d}	8.6^{d}	11.3^{f}	13.4^{d}	16.7^{e}	11.7^{c}

Means followed by *common letter* are not significantly different at $p \leq 0.05$

Table 2 Rubber yield and biochemical parameters related to latex regeneration in different clones of *Hevea* during peak yielding season (Trial-1)

Clones	Yield (g/tree/tap)	Sucrose (mM)	ATP (μM)	Invertase (nmol glucose liberated/min/mg protein)
RRII 105	91.86[a]	10.9[d]	278.2[a]	113.4[a]
PB 260	79.93[a]	2.6[e]	245.6[b]	105.9[b]
RRIM 600	51.56[bc]	15.2[bc]	253.6[b]	103.4[b]
PB 217	55.00[bc]	25.6[a]	217.4[c]	80.9[c]
GT-1	48.27[bc]	10.3[cd]	263.2[ab]	94.2[bc]
RRII 33	15.64[d]	14.1[bc]	140.0[d]	57.8[d]
RRII 38	25.73[d]	18.0[b]	144.3[d]	59.3[d]

Means followed by *common letter* are not significantly different at $p \leq 0.05$

Table 3 Rubber yield and biochemical parameters related to latex regeneration in RRII 400 series clones of *Hevea* during peak yielding season (Trial-2)

Clones	Yield (g/tree/tap)	Sucrose (mM)	ATP (μM)	Invertase (nmol glucose liberated/min/mg protein)
RRII 414	104.1	5.7	286.9	117.2
RRII 417	107.7	7.8	270.1	100.3
RRII 422	116.6	6.9	284.8	126.1
RRII 429	100.5	7.7	290.9	121.5
RRII 430	114.6	6.1	277.4	130.8
RRII 105	93.3	6.5	257.2	112.8
CD ($p < 0.05$)	16.2	ns	18.6	12.6

CD Critical difference significant at $p < 0.05$, *ns* non significant

Availability of sucrose (sucrose supply) to laticifers and its catabolism by invertase were shown to play a major role in latex regeneration mechanism. Sucrose is converted into mevalonate and then to isoprene units that polymerise to give natural rubber with the consumption of ATP (Jacob et al. 1989). ATP is also the specific substrate for the proton pumping ATPase located on the lutoid membrane (Coupe and Chrestin 1989; Gidrol et al. 1988). Positive and negative correlations between latex yield and sucrose have been reported (Eschbach et al. 1984; Jacob et al. 1985). High sucrose content in latex would be indicating increased loading of sucrose to laticifers. However, it also indicates low metabolic utilization and accumulation of sucrose in the tissues. The present data showed that sucrose level is significantly high (above 25 mM), and ATP and invertase activity were low in clone PB 217 compared to other high yielding clones. In such clones the general metabolism may be slow (Prevot et al. 1986) and these clones may respond well to ethylene stimulation while harvesting latex in every third daily (d3 tapping). Ethylene stimulation is not recommended in this clone during initial years after opening the trees for tapping due to low dry rubber content. In clone PB 260, the sucrose level is very low (2.6 mM) and it showed high ATP and invertase activity and sucrose utilization may be much faster and favors the latex regeneration processes. The medium yielding clone GT1 which showed a rising yield trend after initial 5 years of tapping and this clone also showed a high level of energy metabolism. In low yielding clones (RRII 33 and RRII 38), sucrose may not be properly metabolized for rubber biosynthesis.

Among the RRII 400 series clones, RRII 414, RRII 422, RRII 429 and RRII 430 recorded higher ATP compared to RRII 105. Invertase activity in latex is significantly high in clones RRII 422 and RRII 430 and these clones showed higher peak season yield compared to RRII 105. No significant difference in sucrose content was observed in these clones. Among the 400 series clones, lowest ATP and invertase activity in latex was observed in clone RRII 417. Similar invertase activities were observed in clones RRII 414 and RRII 429 (Table 3).

Correlation between yield and biochemical parameters related to latex flow and regeneration were studied earlier in different rubber clones (Eschbach et al. 1984; Jacob et al. 1985; Prevot et al. 1986; Gohet et al. 2003; Simon 2003; Sreelatha 2003; Nair et al. 2001; Sreelatha et al. 2014). Seasonal variations in biochemical parameters viz, ATP, antioxidant enzymes and invertase activity in the C-serum of different *Hevea* clones and correlation of these parameters with rubber yield were also studied (Tupy and Primot 1976; Jacob et al. 1989; Sreelatha et al. 2004, 2009, 2011).

It was already established a positive correlation between rubber biosynthesis, latex ATP and invertase activity in the high yielding rubber clones (Simon 2003; Sreelatha et al. 2004). Availability of large amount of ATP in the laticiferous tissue makes rubber biosynthesis more efficient and

its indirect effect is mediated through lutoid membrane H^+ ATPase and H^+ pyrophosphatase activity which increases the latex pH to favorable levels and activates invertase enzyme in latex (Jacob et al. 1985).

Activity of lutoid membrane enzymes and protein synthesis in latex

ATPase activity of lutoids was significantly high in the latex of RRII 105, PB 260 and RRIM 600. The enzyme activities were comparable in clones PB 217 and GT1. Low yielding clones (RRII 33 and RRII 38) showed very low activity of lutoid membrane ATPase. Pyrophosphatase activity was highest in the clone RRII 105 followed by RRIM 600. Clones PB 260, PB 217 and GT1 had similar activities. Low yielding clones showed very low pyrophosphatase activities compared to high and medium yielders (Table 4).

Among the RRII 400 series clones RRII 414, RRII 422 and RRII 430 showed significantly higher ATPase activities compared to RRII 105. Clones RRII 417 and RRII 429 showed low ATPase activities. Significantly high pyrophosphatase activity was observed in clones RRII 105, RRII 414 and RRII 430. Clones RRII 417, RRII 422 and RRII 429 showed similar activities (Table 5). Continuous supply of ATP is needed to maintain the pH gradient and when there is lack of adequate ATP to function ATPase efficiently, the lutoids gradually lost the pool of protons. Lutoid acidification and cytosolic pH regulation are actively controlled by ATPase when availability of its own substrate ATP is optimum (Marin et al. 1981; Chretin 1982). When there is an increase in ATPase activity followed by an increase in its specific substrate (ATP), efficient activation of H^+ ATPase occurs in vivo. This favors the pH optimum for efficient functioning of invertase in the cytosol. Ethylene stimulation of rubber trees also activates tonoplast H^+ pumping ATPase (Gidrol et al. 1988). In the laticiferous tissue, the H^+ pyrophosphatase joins with ATPase to regulate the cell pH by keeping the lutoid stable and maintain an optimum condition for latex regeneration (Siswanto et al. 1994).

Protein biosynthetic capacity was highest in the clone PB 260, indicating efficient and fast regeneration of cellular materials lost during the previous harvesting of latex. Clone RRIM 600 and GT1 also showed a higher protein

Table 4 Lutoid membrane enzymes and protein synthesis in different clones of *Hevea* during peak yielding season (Trial-1)

Clones	H^+ ATPase (μM Pi liberated/min/mg protein)	H^+ pyrophosphatase (μM Pi liberated/min/mg protein)	Protein biosynthetic capacity (cpm/min/mg protein)	
			Square root transformation values	Original mean
RRII 105	6.97[a]	12.62[a]	71.3[c]	5110
PB 260	6.45[a]	8.97[c]	98.1[a]	9649
RRIM 600	6.3[a]	10.78[b]	90.3[a]	8316
PB 217	5.34[b]	9.18[c]	83.0[b]	6730
GT-1	5.15[b]	8.79[c]	89.0[a]	7969
RRII 33	3.76[c]	5.80[d]	67.3[c]	4489
RRII 38	3.49[c]	6.88[d]	64.1[c]	4117

Means followed by *common letter* are not significantly different at $p \leq 0.05$

Table 5 Lutoid membrane enzymes and protein synthesis in RRII 400 series clones of *Hevea* during peak yielding season (Trial-2)

Clones	H^+ ATPase (μM Pi liberated/min/mg protein)	H^+ pyrophosphatase (μM Pi liberated/min/mg protein)	Protein biosynthetic capacity (cpm/min/mg protein)	
			Square root transformation value	Original mean
RRII 414	6.23	12.83	84.8	7222
RRII 417	3.21	10.21	72.4	5251
RRII 422	6.43	10.98	76.0	5778
RRII 429	3.03	10.36	83.7	7017
RRII 430	6.81	12.93	86.1	7396
RRII 105	5.16	13.07	70.6	4994
CD ($p < 0.05$)	1.02	1.23	11.0	

CD Critical difference significant at $p < 0.05$

biosynthetic capacity compared to low yielders. The clones RRII 414, RRII 429 and RRII 430 showed higher protein synthesis compared to RRII 105. Protein biosynthetic capacity of clones RRII 417 and RRII 422 is comparable to RRII 105 (Tables 4, 5). A high rate of metabolic activity is required for energy generating catabolic pathways (glycolysis) and anabolic processes such as reconstitution of proteins and nucleic acids lost during tapping. The synthesis and turnover of proteins are also very important in latex regeneration mechanism and overall latex production capacity of the clones. Activation of protein synthesis was also observed in latex after stimulating the trees with ethephon (Coupe and Chrestin 1989).

Clone characteristics

RRII 105 is characterized as a high metabolic clone which showed high ATP, invertase, ATPase and pyrophosphatase activities. This clone responds well to stimulation using ethephon under S/2 d3 6d/7 tapping system (Rajagopal et al. 2004). In clone PB 260 better utilization of sucrose is evidenced by increased latex ATP and invertase activity and very low level of latex sucrose. This clone also showed a high protein biosynthetic capacity which favors efficient regeneration of latex before the next tapping. Clone PB 217 is characterized by very high sucrose, low ATP and invertase activity and may respond well to stimulation as these are the earliest biochemical changes induced by stimulation (Coupe and Chrestin 1989; Zhu and Zhang 2009). RRIM 600 showed high sucrose, ATP and protein biosynthesis. GT1 which is a medium yielding clone is characterized by high level of ATP and protein synthesis. Low yielding clones RRII 38 and RRII 33 showed a very low level of ATP, ATPase, pyrophosphatase activities and protein biosynthesis in latex. High sucrose level noticed in these clones may be due to slow metabolic utilization of sucrose for rubber biosynthesis. Sucrose may be accumulating and not metabolized properly for rubber synthesis. These clones do not respond to ethylene stimulation also. Among the RRII 400 series clones, RRII 414, RRII 422 and RRII 430 showed high ATP, invertase and ATPase activities. RRII 417 recorded very low ATPase and protein biosynthetic capacity. This clone may respond well to stimulation as both these parameters were induced by ethylene (Coupe and Chrestin 1989). RRII 429 is a clone with high energy metabolism and protein synthesis but lutoid membrane enzyme activities were low. The carbohydrate metabolism, membrane transport mechanism and protein biosynthetic capacity of different clones showed variations and this study on latex regeneration would be useful for selecting the most suitable harvesting systems and stimulation methods for these clones. Response to different tapping systems and stimulation intensity varies from clone to clone. This result will help to optimize latex production by choosing the most appropriate latex harvesting methods such as high or low frequency tapping systems and ethylene stimulation based on the latex regeneration capacity of each rubber clone.

Conclusion

Variations in biochemical profiles and latex regeneration mechanism in twelve different clones of *Hevea* with varying yield potentials were studied. Significant differences were observed among the clones for biochemical composition, enzyme activities and protein biosynthetic capacity. These critical parameters associated with latex regeneration are useful in characterizing clones with high or low metabolic groups. In the present scenario of labor shortage for tapping rubber trees in India and high cost of production, ethylene stimulation is recommended for increasing latex production under low frequency tapping systems. The metabolic profiling studies would be useful for selecting suitable latex harvesting systems and stimulation methods to different clones including the newly released clones of RRII 400 series for obtaining a sustainable rubber yield.

Author contribution statement Sreelatha. S and Sheela P. Simon: designed the experiment, conducted research and wrote the paper. V. C. Mercykutty and Kavitha K. Mydin: helped in clone selection and involved in discussion of the results and should be considered as equal authors. Krishnakumar, R, Annamalainathan, K and James Jacob: contributed in research concept and discussion of results. All the authors have read and approved the manuscript in its final form.

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