BIOCHEMICAL CHANGES ASSOCIATED WITH LATEX PRODUCTION UNDER LOW FREQUENCY TAPPING IN *HEVEA BRASILIENSIS*

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Received: 01 January 2019 Accepted: 03 April 2019

Sreelatha, S., Thomas, K.U., Rajagopal, R., Karunaichamy, K., Simon, S.P., Annamalainathan, K., and Jacob, J. (2019). Biochemical changes associated with latex production under low frequency tapping in *Hevea brasiliensis*. *Rubber Science*, **32**(1): 42-52.

Latex production in *Hevea brasiliensis* can be optimized by appropriate tapping frequency and yield stimulation using ethephon. High frequency tapping and non-judicious use of yield stimulant may cause oxidative stress to the laticiferous system and leads to severe metabolic disorders like tapping panel dryness (TPD). In the present study, low frequency tapping systems with different levels of yield stimulation were tested in the clone RRII 105 for rubber yield. Biochemical parameters related to latex production and oxidative stress indicators were also studied. After four years of tapping, the cumulative yield under low frequency tapping systems was at par with S/2 d2 tapping frequency except S/2 d3 frequency without stimulation. TPD incidence was more than 21 per cent under high frequency (S/2 d2) tapping without yield stimulation. Under weekly tapping (S/2 d7), TPD incidence was only six per cent with the highest rounds of annual ethephon application. No significant stress effects were observed subsequent to various stimulation schedules under S/2 d4, S/2 d6 and weekly tapping as indicated by higher levels of invertase, ATP, protein synthesis, optimum thiol content, high utilization of sucrose and non-accumulation of stress indicators like proline and phenol. Sustainable high rubber yield obtained under weekly tapping without any stress effects due to frequent ethephon stimulation and low incidence of TPD suggests that S/2 d7 tapping system as the best option for rubber growers for harvesting latex from rubber trees to solve the current problems of low rubber price, scarcity of labour and high cost of production.

Key words: Ethephon stimulation, *Hevea brasiliensis*, Latex production, Low frequency tapping, Rubber yield

INTRODUCTION

Latex production in *Hevea brasiliensis* depends mainly on the availability of sucrose and biochemical energy for rubber biosynthesis and tolerance of the laticiferous system to various stresses including tapping and ethephon stimulation (Jacob *et al.*, 1997). Choosing appropriate tapping system and stimulation schedule are essential factors to get sustainable long term rubber yield and to maintain the optimum physiological status of rubber trees. High frequency

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tapping and excessive stimulation may cause oxidative stress to the laticiferous system and leads to more tapping panel dryness (Chrestin *et al.*, 1986; Das *et al.*, 2002). Ethephon stimulation increases duration of latex flow and production after tapping by activating laticifers metabolism and delay latex coagulation (Coupe and Chrestin, 1989). Stimulation effect may also vary with rubber clones (Gohet *et al.*, 1995). Earlier data indicated that under high frequency tapping (S/2 d2) incidence of tapping panel dryness (TPD) was high in clone RRII 105 compared to low frequency tapping (S/2 d3) with appropriate stimulation (Karunaichamy *et al.*, 2001; Rajagopal *et al.*, 2004). Low frequency tapping systems for reduction in cost of production was reported from India for many clones (Rubber Board, 2002; Vijayakumar *et al.*, 2001; Vijayakumar, 2017; Rajagopal *et al.*, 2003; Karunaichamy *et al.*, 2012) and from other rubber growing countries (Rodrigo, 2007; Kudaligama *et al.*, 2010; Soumahin *et al.*, 2010; Sainoi *et al.*, 2017).

Large scale studies conducted at various rubber plantations and a comprehensive study on low frequency tapping at Harrison Malayalam Ltd., (HML), Koney estate showed that the cumulative yield of clone RRII 105 over four years of tapping under different low frequency tapping systems with stimulation were on par with S/2 d2 frequency except S/2 d3 without stimulation (Thomas, 2017). In the present scenario of continuing low rubber price, tapper shortage, high cost of production and climate variability, low frequency tapping system is a viable option for rubber growers. However, it needs more number of stimulations to get sustainable high yield. Higher round of stimulant application is a concern for the growers whether it affects the health of the tree and increasing the incidence of TPD. The objective of this study was to assess the stress effects if any, caused by frequent ethephon application under low frequency tapping systems in the popular rubber clone RRII 105 as detailed investigation on this aspect is very meagre.

MATERIALS AND METHODS

Experiment details

The study was carried out at, Koney estate, Harrisons Malayalam Ltd., Pathanamthitta District, Kerala ($9^0$13′ 35.98"N and 76^0 50′ 59.21"E) with newly opened trees of clone RRII 105. The design of the experiment was completely randomized block replicated three times with a total of 24 plots. All the trees were rain guarded and tapped throughout the year without any summer rest. Eight treatments (high and low frequency tapping systems) *viz*. T1- S/2(RG) d2 6d/7, T2- S/2(RG) d3 6d/7 , T3-S/2(RG) d3 7d/7. ET2.5% Pa 2/y, T4- S/2(RG) d3 6d/7. ET2.5% Pa 3/y,T5- S/2(RG) d4 7d/7. ET2.5% Pa 4/y, T6- S/2(RG) d4 6d/7. ET2.5% Pa 6/y, T7- S/2(RG) d6 7d/7. ET2.5% Pa 10/y, T8- S/2(RG) d7 6d/7. ET2.5% Pa 12/y.

Yield recording was done from 300 trees per treatment. The treatment details are given as per the new tapping notations (Vijayakumar *et al.*, (2009). Stimulation was carried out using 2.5 per cent Ethephon (ET) applied on the tapping panel following panel application method (Rajagopal *et al.*, 2002). Under d6 and d7 frequency of tapping (treatments T7 & T8), 20 and 24 rounds of ethephon application per year was carried out during initial two years. Yield from each plot was recorded as latex and scrap for all tapping days. Dry rubber content (DRC) of each plot was also determined and the dry rubber yield was expressed as kg 400 trees^{-1}.

For biochemical analysis, a total of 144 trees (18 trees per treatment) having comparable girth and latex yield were selected. The biochemical study was commenced from May

2011 onwards. Latex samples were collected from each tree during May 2011 and peak yielding season (August, September, October and November) for three consecutive years (2011-2013).

Latex collection and preparation of C-serum

The initial 5 ml latex flowing immediately after tapping was discarded and the subsequent latex was collected in vials in an ice bath and used for biochemical estimations. For invertase enzyme activity and *in vitro* protein biosynthetic studies, 4 ml latex was mixed with equal volume of stabilizing buffer (100 mM Tris-HCl (pH 8.0), 40 percent glycerol, 4 mM glutathione, 0.1 percent sodium azide) and centrifuged at 4°C for 45 min (59000xg, Sorvall OTD 55B Ultracentrifuge). Clear layer of C-serum was separated and used for measuring protein biosynthesis and enzyme assay (Gidrol and Chrestin, 1984).

Extraction and estimation of biochemical parameters

ATP concentration in latex was determined according to Amalou *et al*. (1992) with certain modifications. ATP was extracted from fresh latex samples (1gm) by treating with 2.5 per cent trichloroacetic acid (TCA) and quantified in a Stratech luminometer using ATP bioluminescent assay kit (FL-AA-Sigma Chemical Company, USA) which contain luciferin-luciferase enzyme. ATP concentration of the whole latex was expressed as μM.

For estimating sucrose and thiols, 1gm latex was extracted using 2.5 per cent TCA and quantified following the procedure of Scott and Melvin (1953); Boyne and Ellman (1972) and expressed as mM.

For phenol estimation, 1gm latex was extracted using 80 per cent alcohol and measured following the procedure of Swain and Hillis (1959) and expressed as mM. For the estimation of proline, 1gm latex was extracted using 3 per cent sulphosalycylic acid and measured according to the procedure of Bates *et al*. (1973) and expressed as mM.

Clear cytosol (C-serum) after centrifugation was used for the determination of invertase activity (Tupy, 1973) with certain modifications. The activity of invertase (EC 3.2.1.26) was expressed as nmol glucose liberated per min^{-1} mg $protein^{-1}$. Protein content was estimated following Lowry *et al.* (1951). *In vitro* protein synthesis was measured in C-serum (Gidrol and Chrestin, 1984) using a mixture of ^{14} C labeled aminoacids (arginine, leucine, lysine and phenyl alanine) and other co factors. The samples were incubated for four hours at room temperature and stopped the reaction by adding 1ml of cold (unlabeled) amino acid mixture (100 mM arginine, leucine, lysine and phenyl alanine) and 1mg ml^{-1} cycloheximide. Proteins were precipitated with 5 per cent TCA and washed three times with 5 per cent TCA, 50 mM KCl and 10mM of each unlabeled aminoacid. The precipitated protein was dissolved in 0.5N NaOH, kept overnight and measured the radioactivity using a Liquid scintillation counter (LKB). The data were analysed using ANOVA and the mean values between treatments were compared using DMRT.

RESULTS AND DISCUSSION

The mean dry rubber yield of four years (kg 400 $trees^{-1}$) under different tapping systems was at par except T2 (S/2 d3 6d/7) without yield stimulation where it was the lowest (Table 1). Annual average dry rubber content among treatments ranged from 29.9-41.6 per cent (d2 to d7). Four years cumulative TPD incidence was more than 21 per cent under d2 (T1) without stimulation whereas it was lower in low frequency tapped trees (T3-T8). Under weekly tapping (T8) TPD was only

Table 1. **Dry rubber yield (kg 400 trees^{-1}) under different tapping frequencies and stimulation (April 2011 to March 2015)**

Treatments	2011-2012	2012- 2013	2013- 2014	2014- 2015	Mean yield of four years
T1- S/2(RG) d2 6d/7	2090^{a}	2188^{a}	2124^{ab}	2034^{c}	2109^{a}
T2- S/2(RG) d3 6d/7	1671^{c}	1663^{b}	1949^{b}	1626^{d}	1727^{b}
T3- S/2(RG) d3 7d/7. ET2.5% Pa 2/y	2123^{a}	2220^{a}	2135^{ab}	2048^{c}	2132^{a}
T4- S/2(RG) d3 6d/7. ET2.5% Pa 3/y	1939^{ab}	1976^{a}	2238^{a}	2054^{c}	2052^{a}
T5- S/2(RG) d4 7d/7. ET2.5% Pa 4/y	1980^{ab}	1937^{a}	2313^{a}	2162^{abc}	2098^{a}
T6- S/2(RG) d4 6d/7. ET2.5% Pa 6/y	1857^{bc}	2109^{a}	2278^{a}	2219^{ab}	2113^{a}
T7- S/2(RG) d6 7d/7. ET2.5% Pa 10/y	1909^{abc}	2085^{a}	1942^{b}	2081^{bc}	2004^{a}
T8-S/2(RG) d7 6d/7. ET2.5% Pa 12/y	1843^{bc}	2017^{a}	2213^{a}	2254^{a}	2082^{a}

Means followed by common letters are not significantly different at p≤0.05

6 per cent with more rounds of ethephon application. Detailed economic analysis of this data on low frequency tapping trial showed that the benefit cost ratio under d2 frequency (T1) was 5.8 whereas it was double under d7 (T8) weekly tapping (Table 2). Bark consumption was the lowest in trees under low frequency tapping (d7) and economic life of trees will be the highest (Thomas, 2017).

Advantage of low frequency tapping (LFT) systems with different levels of stimulation in different clones for reduction in cost of production was reported earlier (Vijayakumar *et al*., 2001; 2017; Rajagopal *et al*., 2003; Karunaichamy *et al*., 2012; Kudaligama *et al*., 2010; Obouayeba *et al*., 2009; Soumahin *et al*., 2010, Sainoi *et al*., 2017). Similarly low incidence of tapping panel dryness was reported under low frequency tapping systems compared to high frequency tapping systems (Sivakumaran and Hashim, 1986; Karunaichamy *et al*., 2001; Rajagopal *et al*., 2003; Obouayeba *et al*., 2009).

In the present study, to characterize the physiological status of trees, different

Table 2. **Dry rubber content, Benefit cost ratio and tapping panel dryness (TPD) status under different frequencies of tapping with stimulation**

Treatments	No. of tapping days	Tapping panel and year	Dry rubber content (%)	Benefit cost ratio	TPD (%)
T1 S/2(RG) d2 6d/7	150	BO-2 (2)	29.9	5.70	21
T2 S/2(RG) d3 6d/7	104	BO-2(1)	36.0	6.85	13
T3 S/2(RG) d3 7d/7. ET2.5% Pa 2/y	121	BO-2(1)	33.8	7.07	11
T4 S/2(RG) d3 6d/7. ET2.5% Pa 3/y	104	BO-2(1)	36.5	7.84	6
T5 S/2(RG) d4 7d/7. ET2.5% Pa 4/y	91	BO-2(1)	36.7	9.10	9
T6 S/2(RG) d4 6d/7. ET2.5% Pa 6/y	78	BO-1(5)	38.6	10.00	10
T7 S/2(RG) d6 7d/7. ET2.5% Pa 10/y	60	BO-1(5)	39.5	11.06	7
T8 S/2(RG) d7 6d/7. ET2.5% Pa 12/y	52	BO-1(5)	41.6	12.35	6

BO-1 first panel on virgin bark of the base panel; BO-2 second panel on virgin bark of base panel

biochemical parameters related to latex production such as sucrose, invertase activity, biochemical energy availability (ATP), protein synthesis and stress indicators *viz*. thiol, phenol and proline were analyzed and the results are presented in Tables 3-8. Sucrose content of latex in trees without stimulation (T1 &T2) was comparable at the beginning (May 2011) and end of the experiment (11-13 mM). During May 2011, treatments T5-T8 showed low latex sucrose as there were two, two, six and 10 rounds of stimulations were already over before starting the biochemical studies. Comparable sucrose level was observed in treatments T3, T4 and T5. Level of sucrose was low in d4 and d6 frequency tapped trees with stimulation (T6 & T7) compared to unstimulated trees (Table 3). Interestingly, under weekly tapping (T8), sucrose content in latex was very low (below 3 mM) and maintained this level throughout the experimental periods (Table 3).

Latex ATP concentration was significantly high in all the low frequency tapped trees with stimulation compared to unstimulated d2 and d3 (T1 & T2) tapped trees (Table 4). The highest ATP concentration was observed

Table 3. **Status of sucrose in latex under different frequencies of tapping with stimulation**

Treatments	May 2011	Sucrose (mM) Peak season (August-November)			Mean of three yrs
		2011	2012	2013	
T1- S/2(RG) d2 6d/7	11.9^{a}	9.26^{ab}	11.13^{a}	10.35^{b}	10.25^{a}
T2- S/2(RG) d3 6d/7	10.7^{ab}	10.31^{a}	8.14^{b}	13.83^{a}	10.76^{a}
T3- S/2(RG) d3 7d/7. ET2.5% Pa 2/y	9.8^{abc}	6.78^{bc}	7.52^{b}	6.49^{d}	6.93^{b}
T4- S/2(RG) d3 6d/7. ET2.5% Pa 3/y	7.9^{bcd}	4.94^{cd}	7.04^{b}	8.96^{bc}	6.98^{b}
T5- S/2(RG) d4 7d/7. ET2.5% Pa 4/y	6.6^{cde}	5.42^{c}	5.41^{cd}	7.36^{cd}	6.07^{b}
T6- S/2(RG) d4 6d/7. ET2.5% Pa 6/y	5.0^{de}	4.25^{cd}	4.89^{de}	3.98^{e}	4.37^{c}
T7- S/2(RG) d6 7d/7. ET2.5% Pa 10/y	4.9^{de}	3.82^{cd}	3.36^{e}	2.89^{e}	3.36^{c}
T8- S/2(RG) d7 6d/7. ET2.5% Pa 12/y	2.8^{e}	2.14^{d}	2.89^{e}	2.99^{e}	2.67^{d}

Means followed by common letters are not significantly different at p≤0.05

Table 4. **Status of ATP in latex under different frequencies of tapping with stimulation**

Treatments	May 2011	ATP (μM) Peak season (August-November)			Mean of three years
		2011	2012	2013	
T1- S/2(RG) d2 6d/7	211.8	247.0^{c}	259.6^{c}	250.0^{c}	252.0^{c}
T2- S/2(RG) d3 6d/7	192.0	242.3^{c}	231.2^{bc}	249.1^{c}	240.6^{c}
T3- S/2(RG) d3 7d/7. ET2.5% Pa 2/y	187.8	301.2^{a}	292.8^{b}	304.2^{a}	299.2^{a}
T4- S/2(RG) d3 6d/7. ET2.5% Pa 3/y	182.4	314.5^{a}	282.4^{ab}	310.6^{a}	302.6^{a}
T5- S/2(RG) d4 7d/7. ET2.5% Pa 4/y	190.9	311.3^{a}	306.8^{a}	324.8^{a}	314.5^{a}
T6- S/2(RG) d4 6d/7. ET2.5% Pa 6/y	191.9	324.0^{a}	331.2^{a}	313.9^{a}	323.9^{a}
T7- S/2(RG) d6 7d/7. ET2.5% Pa 10/y	200.5	269.6^{b}	296.1^{ab}	274.1^{b}	279.9^{b}
T8- S/2(RG) d7 6d/7. ET2.5% Pa 12/y	207.5 NS	296.3^{b}	270.8^{b}	247.9^{b}	271.6^{b}

Means followed by common letters are not significantly different at p≤0.05

Table 5. **Invertase activity and protein biosynthetic capacity in latex C-serum during peak yielding season (August-November 2013)**

Treatments	Invertase activity (μM glucose liberated $min^{-1}mg$ $protein^{-1}$)	Protein synthesis (cpm mg $protein^{-1}$)	
		Square root transformed values	Mean values
T1- S/2(RG) d2 6d/7	180.9^{e}	78.5^{c}	6177
T2- S/2(RG) d3 6d/7	220.6^{d}	79.8^{c}	6375
T3- S/2(RG) d3 7d/7. ET2.5% Pa 2/y	248.1^{d}	84.8^{b}	7204
T4- S/2(RG) d3 6d/7. ET2.5% Pa 3/y	196.7^{e}	84.6^{b}	7176
T5- S/2(RG) d4 7d/7. ET2.5% Pa 4/y	296.1^{c}	92.5^{a}	8569
T6- S/2(RG) d4 6d/7. ET2.5% Pa 6/y	283.4^{c}	94.5^{a}	8946
T7- S/2(RG) d6 7d/7. ET2.5% Pa 10/y	381.2^{b}	85.4^{b}	7312
T8- S/2(RG) d7 6d/7. ET2.5% Pa 12/y	401.8^{a}	87.6^{b}	7691

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

in d4 (T5 & T6) with four and six rounds stimulation per year. T7 & T8 also have significantly high ATP content than unstimulated control. Significantly high invertase activity was observed in all low frequency tapped trees with stimulation compared to S/2 d2 and S/2 d3 (T1-T4). Highest invertase activity was observed in T8 (weekly tapping). Compared to unstimulated trees, protein biosynthetic capacity was higher in all low frequency tapped trees with stimulation (Table 5). Protein synthesis rate was the highest in T5 and T6 (S/2 d4).

Sucrose supply and its catabolism by invertase and ATP availability were shown to play a major role in latex regeneration mechanism between two tappings (Jacob *et al.*, 1997). Positive correlation between rubber yield, latex ATP and invertase activity in clone RRII 105 and effect of stimulation on low frequency tapped trees after tapping rest for different periods were studied earlier (Sreelatha, 2003; Simon, 2003). In the present study very low level of sucrose content (less than 3 mM) and high ATP, invertase activity and efficient protein biosynthetic capacity reveals that latex regeneration process was geared up intensively after frequent stimulation under weekly tapping (T8) and the sucrose loading and utilization is maintained throughout the experimental period (three consecutive years). Sucrose level in unstimulated trees under S/2 d2 (T1) and S2 d3 (T2) was recorded in the range of 11-13 mM. In all the low frequency tapped trees with stimulation sucrose was utilized effectively after stimulation. Carbohydrate metabolism, availability of ATP and activities of many metabolic enzymes have been shown to be regulated by ethylene in rubber trees (Tupy 1973; Tupy and Premot 1976; Gidrol *et al.*, 1988; Coupe and Chresin 1989; Amalou *et al.*, 1992).

Ethylene stimulation also modifies the balance between supply and demand of sucrose in bark and increased sucrose transport into laticifers (Silpi *et al.*, 2006; Dustoit-Coucaud *et al.*, 2009). Activation of protein synthesis after stimulating the trees with ethephon was reported by Coupe and Chrestin, (1989). Synthesis and turnover of proteins are very important in latex regeneration mechanism and overall latex production capacity of the clones. Protein biosynthetic capacity of different clones were

Table 6. **Status of Thiol in latex under different frequencies of tapping with stimulation in clone RRII 105**

Treatments	May 2011	Thiols (mM) Peak season (August-November)			Mean of 3 years
		2011	2012	2013	
T1- S/2(RG) d2 6d/7	0.29	0.22^{b}	0.327^{a}	0.379^{a}	0.31^{a}
T2- S/2(RG) d3 6d/7	0.28	0.20^{b}	0.322^{a}	0.263^{ab}	0.26^{b}
T3- S/2(RG) d3 7d/7. ET2.5% Pa 2/y	0.20	0.13^{c}	0.197^{de}	0.211^{c}	0.18^{d}
T4- S/2(RG) d3 6d/7. ET2.5% Pa 3/y	0.26	0.30^{a}	0.289^{abc}	0.271^{b}	0.29^{a}
T5- S/2(RG) d4 7d/7. ET2.5% Pa 4/y	0.28	0.19^{b}	0.256^{bc}	0.255^{ab}	0.23^{c}
T6- S/2(RG) d4 6d/7. ET2.5% Pa 6/y	0.25	0.22^{b}	0.296^{ab}	0.365^{a}	0.29^{a}
T7- S/2(RG) d6 7d/7. ET2.5% Pa 10/y	0.18	0.18^{c}	0.275^{b}	0.242^{ab}	0.23^{c}
T8- S/2(RG) d7 6d/7. ET2.5% Pa 12/y	0.24	0.28^{b}	0.236^{cd}	0.286^{b}	0.24^{bc}
	NS				

Means followed by common letters are not significantly different at p≤0.05

studied earlier and found that high metabolic clones have higher protein biosynthetic capacity (Sreelatha *et al.*, 2016).

Though ethephon stimulation increases metabolic activities related to rubber biosynthesis, it can also induces other stress effects such as increasing the production of toxic oxygen species which leads to oxidative stress to laticiferous system (Coupe *et al.*, 1989). One of the general responses to stress is the activation of synthesis of antioxidants. Thiols in latex act as an antioxidant to prevent the formation of toxic oxygen and involved in activation of several pH dependent enzymes (Eschbach *et al.*, 1984). At the beginning of the experiment there was no significant variation in thiol content between treatments. High thiol level is

Table 7. **Status of Proline in latex under different frequencies of tapping with stimulation in clone RRII 105**

Treatments	May 2011	Proline (mM) Peak season (August-November)			Mean of three years
		2011	2012	2013	
T1- S/2(RG) d2 6d/7	0.61	0.97	1.06	1.33	1.12
T2- S/2(RG) d3 6d/7	0.51	0.76	0.78	1.24	0.93
T3- S/2(RG) d3 7d/7. ET2.5% Pa 2/y	0.58	1.12	0.96	1.04	1.04
T4- S/2(RG) d3 6d/7. ET2.5% Pa 3/y	0.49	0.87	0.91	0.96	0.91
T5- S/2(RG) d4 7d/7. ET2.5% Pa 4/y	0.56	1.03	1.14	1.06	1.08
T6- S/2(RG) d4 6d/7. ET2.5% Pa 6/y	0.62	0.96	0.95	1.07	0.99
T7- S/2(RG) d6 7d/7. ET2.5% Pa 10/y	0.60	1.06	1.11	0.85	1.01
T8- S/2(RG) d7 6d/7. ET2.5% Pa 12/y	0.66	0.69	0.88	1.08	0.88
	NS	NS	NS	NS	NS

Table 8. **Status of Phenol in latex under different frequencies of tapping with stimulation in clone RRII 105**

Treatments	May 2011	Phenol (mM) Peak season (August-November)			Mean of three yrs
		2011	2012	2013	
T1- S/2 (RG) d2 6d/7	0.66	0.75	1.01^{cd}	1.20	0.99^{c}
T2- S/2 (RG) d3 6d/7	0.60	0.76	1.27^{abc}	1.48	1.17^{abc}
T3- S/2 (RG) d3 7d/7. ET2.5% Pa 2/y	0.89	0.90	1.12^{bcd}	1.58	1.23^{ab}
T4- S/2 (RG) d3 6d/7. ET2.5% Pa 3/y	0.61	0.74	1.22^{bcd}	1.43	1.13^{abc}
T5- S/2 (RG) d4 7d/7. ET2.5% Pa 4/y	0.93	0.75	1.37^{ab}	1.22	1.12^{abc}
T6- S/2 (RG) d4 6d/7. ET2.5% Pa 6/y	0.70	0.78	1.03^{d}	1.20	1.01^{c}
T7- S/2 (RG) d6 7d/7. ET2.5% Pa 10/y	0.77	0.82	1.25^{ab}	1.48	1.18^{a}
T8- S/2 (RG) d7 6d/7. ET2.5% Pa 12/y	0.67	0.75	1.11^{bcd}	1.36	1.07^{bc}
	NS	NS		NS	

Means followed by common letters are not significantly different at p≤0.05

maintained at the end of the experiment under d4 and d7 systems of tapping with six and 12 rounds of stimulation per year (T6 and T8). Very low thiol level was noticed in trees under d3 with 2 rounds of stimulation per year compared to unstimulated d3 tapped trees (Table 6).

Phenols are known antioxidants involved in cellular responses and triggered to resist oxidative stress (Mittler, 2002). Accumulation of proline in plant tissues is a common mechanism to various forms of physiological stresses. Both unstimulated and stimulated trees accumulated proline and phenol due to cumulative effect of tapping and stimulation stress at the end of the experiment. However, between high and low frequency tapped trees, no significant difference was noticed (Table 7 & 8). Excessive withdrawal of latex after stimulation may probably leads to physiological stress in the tapping panel, but in the present study the content of these biomolecules is increasing over the years, but no significant difference was observed between treatments. Proline and phenol were not accumulated even after monthly stimulation in weekly tapped trees compared to other treatments. Incidence of tapping panel dryness was also low in weekly tapping system compared to high frequency tapping (Table 2).

CONCLUSION

The study revealed that balanced and activated metabolism in treatments T5 and T6 with optimum sucrose loading, high energy availability (ATP), high rate of protein synthesis and better protection of laticiferous system. Treatment T8 (weekly tapping) showed high utilization of sucrose after stimulation as indicated by very low sucrose, high invertase activity, normal thiol levels and energy availability compared to S/2d2 6d/7, S/2 d36d/7 and S/2 d3 7d/7 with two round of stimulation (T1, T2 & T3). Though the sucrose utilization was more in weekly tapping after monthly stimulation, the level was maintained stably throughout the experimental period. Based on data for three consecutive years, no stress effect on laticiferous system was observed after frequent stimulation under weekly tapping. Increased activation of sucrose catabolism by invertase and high energy availability may be the major

factor determining sustainable latex yield after stimulation in low frequency tapped trees of clone RRII 105. Due to low stress effects, less tapping panel dryness and more economical, weekly tapping system (S/2 d7) seems to be a better option for rubber growers including small holders.

ACKNOWLEDGEMENT

The authors are grateful to Mr. P. Aneesh, Assistant Statistician, Rubber Research Institute of India for analysis of data and all those who are directly or indirectly involved in yield recording and latex collection for biochemical analysis.

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