Studies on Yield and Biochemical Sub-components of Latex of Rubber Trees (Hevea brasiliensis) with a Special Reference to the Impact of Low Temperature in a Non-optimal Environment

BADRE ALAM*#, GITALI DAS**, SHAMMI RAJ**, SANTANU ROY**, T K PAL** AND S K DEY**

This study was conducted to examine the relationship of yield with latex sub-components with a special reference to low temperature effects in five commercially popular clones (GT 1, RRII 105, RRII 118, PB 235, and RRIM 600) of rubber tree Hevea brasiliensis in a non-traditional area of rubber cultivation in North-East India where low temperature prevails in winter There was a clear seasonal and clonal variation in yield. The pre-winter (October to November) was the broad peak yielding period Rubber yield due to low temperature during the winter season (December to February) decreased in the range of about 8% to 40% depending on the clones. Low temperatureinduced yield depression was lowest in clone RRIM 600 which indicated its relatively better acclimatisation to the cold climate Latex thiol content increased about five-fold in the winter season in comparison to the peak yielding pre-winter season. This remarkable increase in thiol content in the latex in the winter season confirmed a positive relation between thiol and cold stress in Hevea for the first time Sugar and thiol were found negatively correlated with yield Positive correlations were obtained between yield and maximum temperature (T_{max}) , minimum temperature (T_{min}) , the total solid content of latex (TSC) and soil moisture (SM) A multiple regression analysis considering the yield as dependent and sugar thiol inorganic phosphate (Pi), TSC, T_{max} , T_{min} and SM as independent variables revealed that about 41% variability of yield can be explained through these independent variables Path coefficient analysis proved that thiol, Pi, TSC and SM have major direct effects on yield while P_1 , TSC, T_{min} and SM were noted to have direct effects on thiol. Strong relations of thiol with yield and minimum temperature showed that the ratio of yield to thiol would be one of the determinants for rubber producing potential of Hevea clones during winter in the non-optimal environment

Key words *Hevea brasiliensis*, yield regulation, cold stress, latex, minimum temperature, soil moisture, sugar, thiol, morganic phosphate

^{*}Plant Physiology Division Rubber Research Institute of India, Kottayam – 686 009 India #Corresponding author (e-mail badre_al@yahoo co in)

Traditionally cultivation of natural rubber (Hevea brasiliensis) has been limited to the humid tropics within 10° north and south latitude where the climate is suitable for its growth In India, rubber is traditionally cultivated in the southern peninsula extending 8° N to 12° N¹ Owing to the growing necessity of natural rubber, Hevea cultivation in India is being expanded to the less and marginally suitable regions of the country including North-East India (22° N to 29 5° N) The physiological constraints for rubber cultivation in different stressful agroclimatic conditions have been recently reviewed² This review has highlighted the adverse affects of abiotic stresses e g drought and low temperature on Hevea in field conditions. It is highly important and necessary to find out suitable Hevea clones to cope with such environmental extremity for successful and sustainable Heyea cultivation For this, comprehensive studies were required to get insights on the response of Hevea trees to the non-optimal environment

This study was conducted in Tripura in North-East India which is known as a nontraditional area of rubber cultivation. As rubber tree is of tropical origin, a wide fluctuations in temperature will affect the in vivo regeneration of latex³⁴ and the yield⁵ Latex synthesis and regeneration after tapping are the key factors for yield regulation^{3 46 9} Latex synthesis and regeneration are mainly controlled by the important components of latex viz sugar, thiol and morganic phosphate¹⁰ Virtually, there are no detailed studies on the effects of prevailing low temperature on yield in Hevea grown under non-optimal conditions in relation to the latex sub-components Therefore, it was hypothesized that such non-optimal conditions will influence the yielding potentials of Hevea clones throughout the year and also the biochemical components of latex will reflect such effects, especially during low temperature

prevailing winter period depending on the intrinsic clonal characteristics

This study, therefore, was undertaken with the following objectives

- To closely monitor the seasonal trends in yield and the biochemical sub-components of latex in different clones in order to identify the high and low yielding seasons with an aim to get insights on the response of Hevea clones to the non-optimal environment
- To examine the relationship between minimum temperature and rubber yield in this agroclimatic zone during prewinter to winter seasons and to study the association of yield with biochemical and environmental factors during this period

MATERIALS AND METHODS

Location, Climatic Details and Plant Materials

This study was conducted at the experimental farm of Regional Research Station of the Rubber Research Institute of India located in Taranagar about 20 km away from Agartala, Tripura (23° 53' N, 91° 15' E, 20 m MSL) in North-East India

The climate of Tripura is sub-tropical This location is of low elevation (20 m MSL) with prolonged winter with low temperature and high to moderate rainfall Peak winter mınımum temperature in Tripura often drops below 10°C11 Meteorological parameters were recorded in our research station's meteorological observatory located a few meters away from the experimental site

The experiment was carried out in the trees planted in 1987 with complete randomised design comprising of five commercially popular Hevea clones viz GT 1, RRII 105, RRII 118, PB 235 and RRIM 600 The total area of the experimental plot was 1 ha The experimental period was from October 1996 to February 1999 To closely monitor the trend of different sub-components of latex throughout the year, weekly analysis of latex was done and presented as the average of three years for the respective weeks The weeks (total 52) were demarcated as per calendar year with respect to the prevailing seasons namely, dry (March to May which consists of 10th - 22nd week), wet (June to September which consists of 23rd – 39th week), pre-winter (October to November which consists of 40th – 48th week) and Winter (December to February which consists of 49th – 9th week)

Yield Recording

The latex was collected on all tapping days. The standard method of yield recording by cup coagulation was carried out by adding a few drops of 1% formic acid followed by drying the coagulate in a smoke house¹². The tapping system adopted was a half-spiral cut with alternate daily harvest (½ S d/2 system). No stimulation was used. The yield is expressed in gram per tree per tapping (gt ½ t ½). The yield recording has also been described elsewhere Following the convention, harvesting of yield was suspended from the 8th to the 13th week. Hevea trees experience heavy defoliation during this period of the year This is the rest period as indicated in the figures.

Biochemical Estimation of the Latex Subcomponents

Every week the latex samples were collected and biochemical analysis were performed

Fresh latex was coagulated with 2 5% trichloro acetic acid and extract was used for biochemical analysis. Anthrone reagent technique was utilised for the estimation of sugar¹⁵. Thiol was estimated following the standard method¹⁶. Inorganic phosphate (Pi) was measured spectrophotometrically¹⁷. Recovery for sugar, thiol and Pi were almost in the range of 95%–98% when estimated after the addition of known amount of standard with the latex extract. For the determination of total solid content (TSC), 10 g of fresh latex sample was dried to a constant weight in a hot air oven at 60°C. There were ten replications per clone for all these estimations.

Soil Moisture Determination

Soil moisture was estimated weekly at 0–30 cm depth with the help of time domain reflectometry probes (Soil moisture Co, USA) at 10 different spots within the plantation. The average moisture content was expressed as percentage basis. The field capacity of the soil was checked using pressure plate techniques and it was found about 24% and the permanent wilting point was about 11%

Statistical Analysis

There were ten replications for each clone (ten trees/clone) for recording yield and biochemical parameters. Average of three years data for the corresponding weeks are presented here. Standard deviations of the means are given in the figures as vertical bars. Detailed statistical analysis eg correlation coefficient matrix, multiple regression analysis taking yield as the dependent and the parameters viz sugar, thiol, iorganic phosphate (P1), TSC, maximum temperature (T_{max}), minimum temperature (T_{min}) and soil moisture (SM) as

independent variables were performed. Path coefficient analysis (standardised partial regression coefficient) was performed to understand the direct and indirect effects of different parameters on the yield and their interrelationships within each other^{18,19}. Since the pre-winter season is the post-monsoon (wet) period immediately followed by the winter season, the detail analysis of interrelationship of different parameters studied were carried out from the data collected throughout the pre-winter to winter season for the three consecutive years of experimentation.

RESULTS

There was a seasonal and clonal variation in yield (*Figure 1*). Among the five clones, PB 235 was the highest and RRII 118 the lowest yielder. The pre-winter season (40th to 48th week) was the peak yielding season in comparison to the other seasons. The percentage reduction in yield (g t⁻¹ t⁻¹) in the winter season in comparison to the peak yielding pre-winter season was the lowest (8%) in RRIM 600 and the highest (40%) in PB 235 indicating a better yielding potential of the former in the cold period compared to the latter. The relationship of different parameters with yield is presented in *Table 1*.

Seasonal and clonal variation in sugar content was noted (*Figure 2*). The high yielding clone, PB 235, had distinctly lower levels of sugar than the other clones. Though there was an increasing trend of sugar content during the low yielding winter period, the sugar content decreased towards the end (5th to 7th week) of the winter season.

Seasonal and clonal variation of thiol was also clearly noted in the present study

(Figure 3). Among the five clones, PB 235, had low level of thiol in latex in contrast to GT 1 and RRII 118. There was about five-fold increase in the thiol content during the winter period in comparison to the other seasons. This increasing trend in thiol coincided with the gradual fall in the minimum temperature (Figure 7 a).

There was a seasonal and clonal variability in Pi (Figure 4). Among the five clones, PB 235, RRII 105 and RRIM 600 showed higher level of Pi in the latex than the other clones. There was an increase in Pi content during the winter period. A clear decreasing trend of TSC was noted during the winter period alongside with the decrease in yield (Figure 5).

It was clear that the locality was mainly dependent on South West monsoon rainfall concentrating during the month of June to September followed by very little rainfall from October to February (40th to 9th week) corroborating with the soil moisture trend (*Figure 7*). The depletion of soil moisture level never reached below permanent wilting point (about 11%) as determined for the soil of the experimental site.

DISCUSSION

Relatively less reduction in yield during the winter period in clone RRIM 600 indicates it as better acclimatised in the cold climate. More reduction in yield in PB 235 during winter season indicates it as relatively cold susceptible. This reduction is attributed to the low temperature stress effects on latex biosynthesis as corroborated with the decline in TSC (Figure 5). Sugar and thiol showed significant negative relation with yield, whereas TSC, T_{max}, T_{min} and SM showed

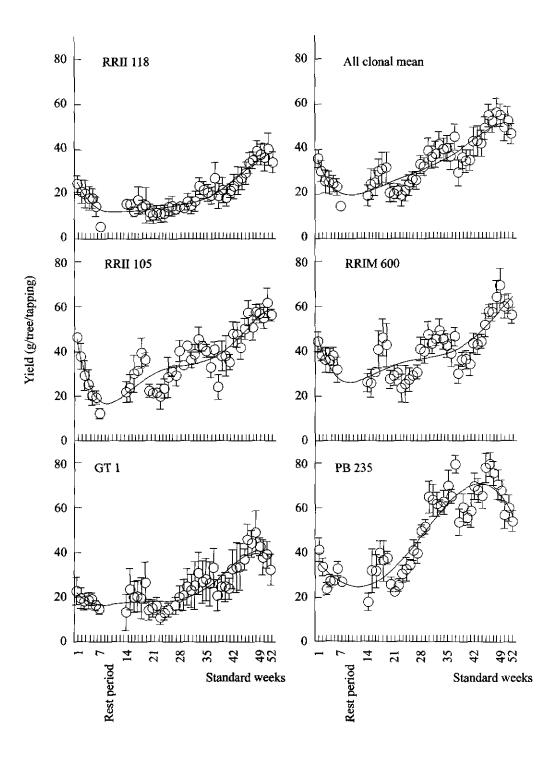


Figure 1. Dry rubber yield (g/tree/tapping) trend of different clones in different weeks in Hevea grown in a non-traditional area of rubber cultivation. Rest period (from 8th to 13th week) means that there was no harvesting of latex during this duration. Vertical bars represent standard deviation (n = 25-30).

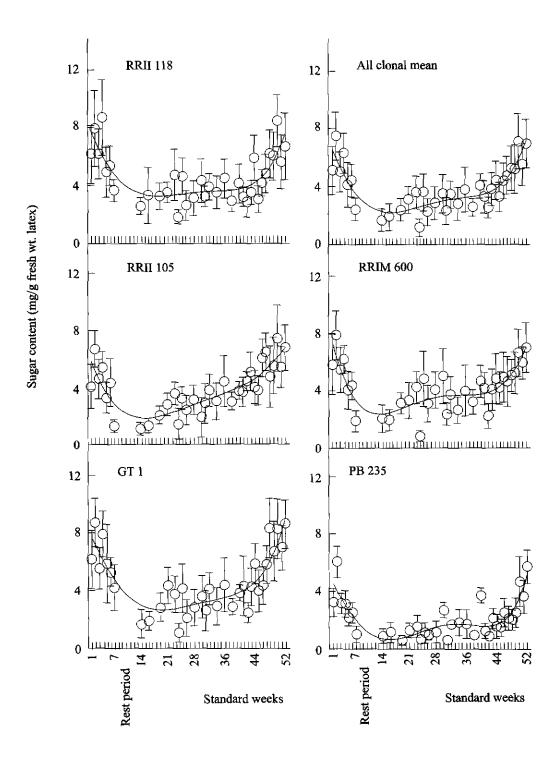


Figure 2. Trend of sugar content (mg/g fresh wt. latex) in latex of different clones in different weeks in Hevea grown in a non-traditional area of rubber cultivation. All the other conditions are same as in Figure 1.

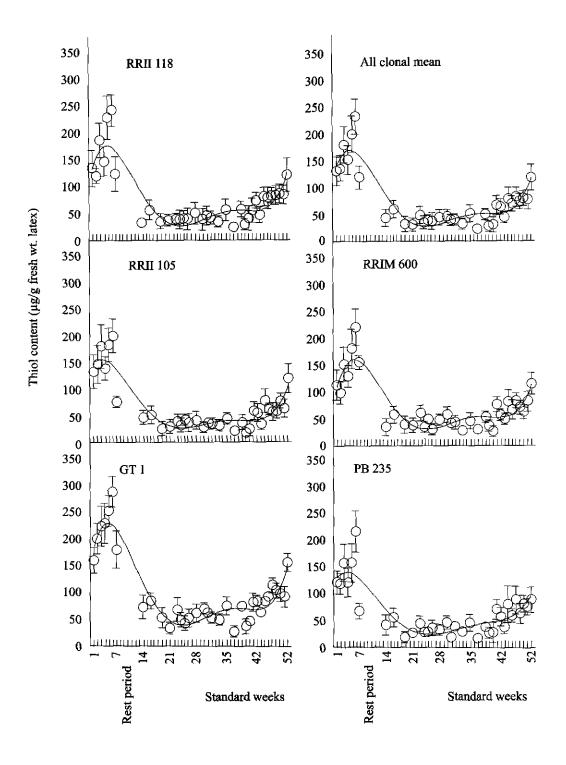


Figure 3. Trend of thiol content (µg/g fresh wt. latex) of latex in different clones in different weeks in Hevea grown in a non-traditional area of rubber cultivation. All the other conditions are same as in Figure 1.

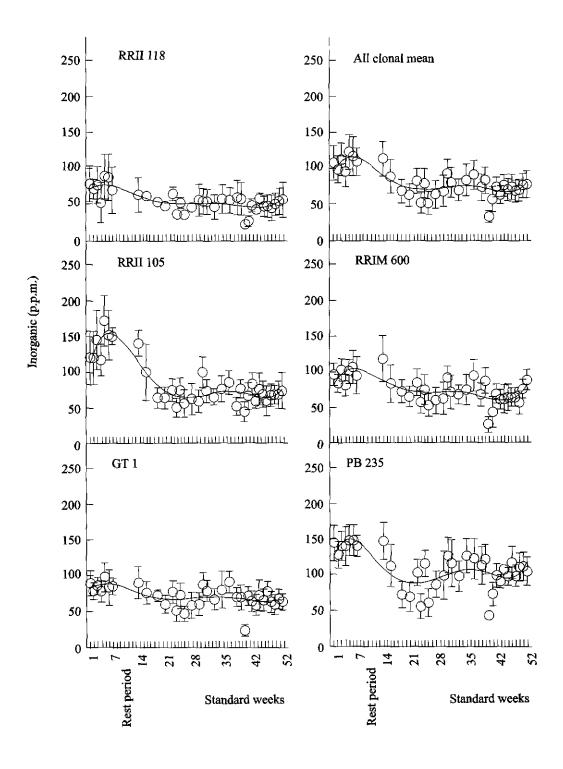


Figure 4. Trend of inorganic phosphorus content (p.p.m.) in latex of different clones in different weeks in Hevea grown in a non-traditional area of rubber cultivation. All the other conditions are same as in Figure 1.

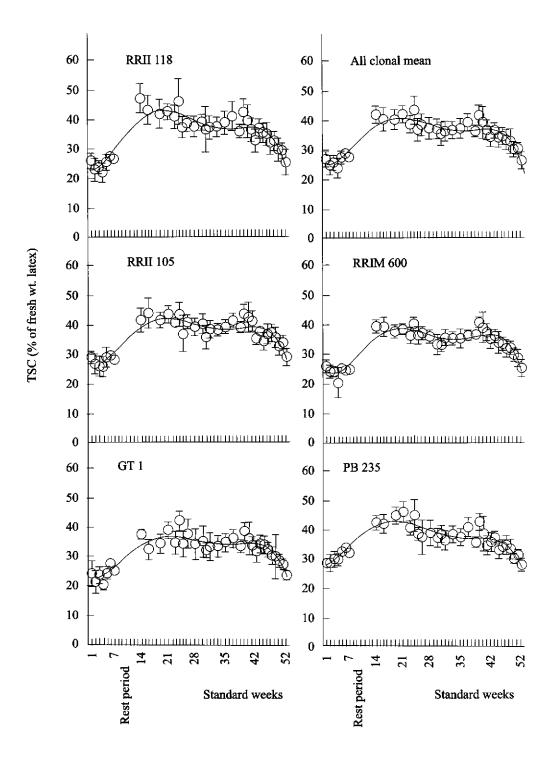


Figure 5. Trend of total solid content [TSC (% of fresh wt. latex)] of latex in different clones in different weeks in Hevea grown in a non-traditional area of rubber cultivation.

All the other conditions are same as in Figure 1.

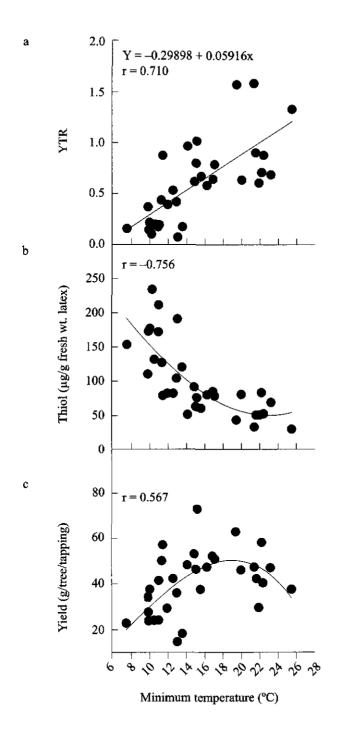


Figure 6. Effect of minimum temperature on YTR (a), thiol (b) and dry rubber yield (c) in Hevea grown in a non-traditional area of rubber cultivation. [YTR (ratio of yield to thiol), thiol (µg/g fresh wt. latex), yield (g/tree/tapping)].

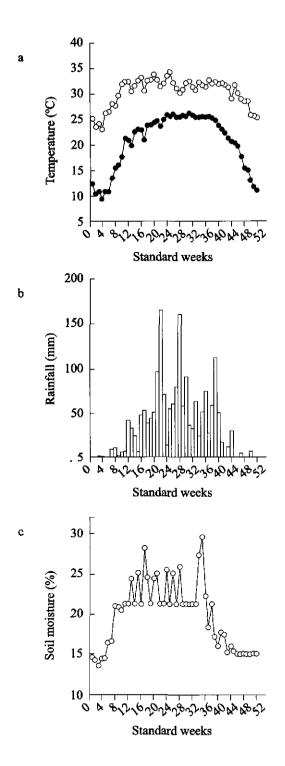


Figure 7. Climatic details [(a: Temperature (°C); b: Rainfall (mm); c: Soil moisture (%)] of the location presented as the weekly average of three years covering the experiment duration.

positive correlation with yield. A multiple regression analysis gave the following equation:

$$Y = 5.154 - 2.621E - 2 * X_1 - 1.322E-1 * X_2 + 1.005E-1 * X_3 + 1.617 * X_4 + 5.662E-1 * X_5 - 6.634 E-1 * X_6 - 9.016 E-1 * X_7 ... 1$$

where Y is yield (g t⁻¹ t⁻¹) and X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 are sugar, thiol. Pi. TSC, T_{max} , T_{min} and soil moisture, respectively. A significant (p<0.01) R^2 (0.414) was obtained which indicates that about 41% of the variability in yield could be explained through these parameters.

The clone PB 235 had distinctly lower levels of sugar than the other clones indicating its better conversion of sucrose to rubber under conducive conditions. Accumulation of sugar is indicative of possible under-utilisation due to low temperature induced inhibition in the metabolic activities 20. The decrease in the sugar content towards the end of the winter season could be due to the cumulative effects of low temperature induced inhibition in photosynthesis²¹. Path analysis (Table 3) revealed that both Tmax and Pi have major direct effects on latex sugar content. Tmax showed negative correlation with sugar which could be due to the increase in respiration which reduced sugar concentration with increase in temperature²².

Leaves of trees contain 95% of thiol as glutathione²³ and thiol plays a very important role in various ways in plant metabolism²⁴. Thiol showed a significant negative relation with yield (*Table 1*). Though the negative correlation between thiol and yield of *Hevea* in the present study is in contrast to an earlier report⁹, similar relationship was also reported²⁰. The high level of thiol content during the

winter period was also noted in *Picea*²⁵. Low temperature-induced yield depression in RRIM 600 was the lowest in spite of having increased thiol content during the cold period. This strongly indicates that this clone has a better cold tolerance potential. As thiols are capable of scavenging toxic free radicals protecting the functions of laticifers and activation of some enzymes^{4,26}, the increasing content of thiol during the winter season (when T_{min} gradually declines to sub-optimal level) in this study indicates the presence of low temperature-induced oxidative stress in *Hevea*.

From this study, it is clear that low temperature-induced accumulation of thiol content has a strong association with yield reduction. Path analysis revealed that it has strong direct effect on yield (Table 2) and indirect effect through TSC. The positive correlation of thiol with Pi has been found through its major direct effects (Table 4) which may be due to the fact that synthesis of glutathione (a major thiol) requires ATPs²⁴ and its synthesis is very tightly controlled27. Positive correlation between thiol and Pi supports the theory that low temperature triggered energy utilisation for thiol synthesis28. Pi showed significant negative correlations with sugar, TSC, T_{max} , T_{min} and SM (Table 1). Significant negative correlation between Pi and sugar strongly leads to speculate a possible role of enzyme sucrose phosphate synthase which acts under allosteric control of Pi29 where Practs as inhibitor.

TSC is an important component of latex because about 90% of TSC is rubber. TSC showed significant positive correlations with yield, T_{max}, T_{min} and SM (*Table 1*). Positive correlation between TSC and yield is in agreement with the reported findings^{20,30}. In this study it is confirmed that soil moisture level was not a limiting factor during the winter period in this agroclimatic zone. Soil

Parameters	Sugar	Thiol	Inorg. P	TSC	Tmax	T _{min}	SM
Thiol	0.242*						
Inorg. P	-0.298*	0.440*					
TSC	-0.474*	-0.647*	-0.220*				
T _{max}	-0.511*	-0.499*	-0.324*	0.771*			
T_{min}	-0.340*	-0.642*	-0.405*	0.772*	0.786*		
SM	-0.280*	-0.402*	-0.184**	0.615*	0.565*	0.731*	
Yield	-0.312*	-0.519*	-0.046	0.521*	0.365*	0.321*	0.144

TABLE 1. CORRELATION COEFFICIENT MATRIX OF DIFFERENT PARAMETERS

TABLE 2. DIRECT (UNDERLINED) AND INDIRECT EFFECTS OF SOME PARAMETERS ON RUBBER YIELD AS DETERMINED THROUGH PATH COEFFICIENT ANALYSIS

Parameters	Sugar	Thiol	Inorg. P	TSC	T _{max}	T_{min}	SM	Total r
Sugar	-0.0030	-0.1077	-0.0522	-0.2152	-0.0517	0.0560	0.0616	-0.312*
Thiol	-0.0007	-0.4453	0.0770	-0.2939	-0.0506	0.1058	0.0883	-0.519*
Inorg. P	0.0009	-0.1959	0.1751	-0.1001	-0.0328	0.0666	0.0405	-0.046
TSC	0.0014	0.2881	-0.0386	0.4543	0.0781	-0.1271	-0.1349	0.521*
T _{max}	0.0015	0.2224	-0.0567	0.3503	0.1012	-0.1295	-0.1240	0.365*
T_{\min}	0.0010	0.2861	-0.0708	0.3507	0.0796	-0.1647	-0.1606	0.321*
SM	0.0008	0.1791	-0.0323	0.2792	0.0572	-0.1205	-0.2196	0.144

^{*} Significant at 1% level. $R^2 = 0.414$.

TABLE 3. DIRECT (UNDERLINED) AND INDIRECT EFFECTS OF SOME PARAMETERS ON SUGAR CONTENT IN LATEX OF HEVEA AS DETERMINED THROUGH PATH COEFFICIENT ANALYSIS

Parameters	Thiol	Inorg. P	TSC	T_{max}	T_{min}	SM	Total r
Thiol	0.1952	-0.2477	0.0492	0.3072	-0.0490	-0.0132	0.242*
Inorg. P	0.0858	-0.5632	0.0167	0.1991	-0.0308	-0.0060	-0.298*
TSC	-0.1263	0.1241	-0.0760	-0.4744	0.0588	0.0201	-0.474*
T_{max}	-0.0975	0.1823	-0.0586	-0.6152	0.0599	0.0185	-0.511*
T_{min}	-0.1254	0.2278	-0.0587	-0.4837	0.0762	0.0239	-0.340*
SM	-0.0785	0.1038	-0.0467	-0.3475	0.0557	0.0327	-0.280*

^{*} Significant at 1% level, $R^2 = 0.530$.

^{*, **} Significant at 1% and 5% level, respectively.

Parameters	Inorg. P	TSC	T _{max}	T _{min}	SM	Total r
Inorg. P	0.2582	0.1229	-0,0849	0.1712	-0.0276	0.440*
TSC	-0.0569	- <u>0.5576</u>	0.2023	-0.3266	0.0918	-0.647*
T_{max}	-0.0836	-0.4299	0.2623	-0.3326	0.0844	-0.499*
T _{min}	-0.1045	-0.4304	0.2063	-0.4231	0.1093	-0.642*
SM	-0.0476	-0.3427	0.1482	-0.3094	<u>0.1494</u>	-0.402*

TABLE 4. DIRECT (UNDERLINED) AND INDIRECT EFFECTS OF SOME PARAMETERS ON THIOL CONTENT IN LATEX OF HEVEA AS DETERMINED THROUGH PATH COEFFICIENT ANALYSIS

moisture was found positively correlated with yield (*Table 1*) as expected 31,32 .

A very consistent increasing trend of the latex thiol content with concomitant gradual fall in minimum temperature and rubber yield led to critically evaluate the role of latex thiol content in order to get mechanistic insights of rubber yield in Hevea at low temperature. Hence it was hypothesized that the ratio of yield to thiol (YTR) would indicate clonal characteristics of Hevea for rubber yield during the winter season. To test this hypothesis, pooled data (yield, thiol and YTR) from all the clones were plotted against minimum temperature (T_{min}) as presented in Figure 6. It was found that T_{min} in the range of 18°C to 20°C was optimum for yield (Figure 6 c). A decreasing trend of thiol was found with the increase in T_{min} (Figure 6 b).

A linear regression (i.e. YTR = $-0.29898 + 0.05916*T_{min}$) between YTR and T_{min} was worked out from the pooled data from the five clones (Figure 6 a). From this equation, a common YTR was calculated as 0.766 for a given value of T_{min} of 18°C. For this, 18°C was chosen because, it was the lower limit of the optimum range (i.e. 18°C to 20°C) of the T_{min} for the yield. Hence 0.766 was taken as the optimum YTR.

Now it was considered that the YTR for an individual clone which will be above the 50% of the optimum YTR (0.766) *i.e.* 0.383, the clone would be considered as relatively better yielding at low temperature. This YTR of 0.383 was taken as a critical value for this experiment. For the individual clones, following linear regression equations between YTR and T_{min} were established, where y = YTR and $x = T_{min}$.

Clones	Equations	
GT 1	y = -0.1711 + 0.0327x (r = 0.711, p< 0.01)	2
RRII 105	y = -0.3929 + 0.0711x (r = 0.616, p< 0.01)	3
RRII 118	y = -0.1375 + 0.0321x (r = 0.615, p<0.01)	4
PB 235	y = -0.9112 + 0.1205x (r = 0.790, p<0.01)	5
RRIM 600	y = -0.0418 + 0.0478x (r = 0.508, p<0.01)	6

To find a critical T_{min} , it was considered that the T_{min} at which the yield is reduced by 50% than the peak yield, would be taken as the

^{*} Significant at 1% level. $R^2 = 0.555$.

critical T_{min} . From Figure 6 c, it was found to be 10°C where yield was 50% of the peak yield (yield obtained at T_{min} of 18°C). Therefore, it is reasonable to accept that the T_{min} of 10°C would be the critical point of low temperature stress on rubber yield. Hence, YTR value was calculated for the individual clones from the above respective equations (2 to 6) for a given T_{min} of 10°C and thus YTR values for the five clones viz. GT1, RRII 105, RRII 118, PB 235 and RRIM 600 were 0.156, 0.318, 0.184, 0.294 and 0.436, respectively. It is seen that among the five clones, YTR value for T_{min} of 10°C for RRIM 600 was 0.436 which is more than the critical value of 0.383. This indicates that RRIM 600 would be relatively better clone for yield during winter season. Furthermore low temperature-induced yield reduction was the lowest (8%) in RRIM 600 than the other clones indicating its relatively better yielding potential in the cold conditions. Higher vielding potential of RRIM 600 in cold climate is in tune with some earlier reports^{33,34}. Therefore, the present study, for the first time, reports that YTR would be one of the determinants to characterise the rubber yielding ability of Hevea clones at low temperature in consideration with other physio-biochemical factors. This information has importance for possible use in latex diagnosis. Furthermore, on the basis of the critical YTR value, stressful minimum temperature could be decided as a cut off point to suspend tapping during winter season. For sustainable yield, this would help avoid additional stress to the trees due to low temperature during the winter period in the non-optimal environment. Moreover, as to date we do not have much detailed information on the physio-biochemical response of Hevea trees to cold climate, this study provides a greater lead towards the better understanding of the responses of Hevea trees in the nonoptimal environment.

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