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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*

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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 temperature-induced 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 Pi, 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; inorganic phosphate

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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° N[1]. 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 reviewed[2]. 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 *Hevea* 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 non-traditional area of rubber cultivation. As rubber tree is of tropical origin, a wide fluctuations in temperature will affect the *in vivo* regeneration of latex[3,4] and the yield[5]. Latex synthesis and regeneration after tapping are the key factors for yield regulation[3,4,6–9]. Latex synthesis and regeneration are mainly controlled by the important components of latex *viz.* sugar, thiol and inorganic phosphate[10]. 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 pre-winter 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[1]. Peak winter minimum temperature in Tripura often drops below 10°C[11]. 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 house[12]. The tapping system adopted was a half-spiral cut with alternate daily harvest ($^1/_2$S.d/2 system). No stimulation was used. The yield is expressed in gram per tree per tapping (gt^{-1} t^{-1}). The yield recording has also been described elsewhere[13]. 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[14]. This is the rest period as indicated in the figures.

Biochemical Estimation of the Latex Sub-components

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 sugar[15]. Thiol was estimated following the standard method[16]. Inorganic phosphate (Pi) was measured spectrophotometrically[17]. 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, U.S.A) 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 *e.g.* correlation coefficient matrix, multiple regression analysis taking yield as the dependent and the parameters *viz.* sugar, thiol, iorganic phosphate (Pi), 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

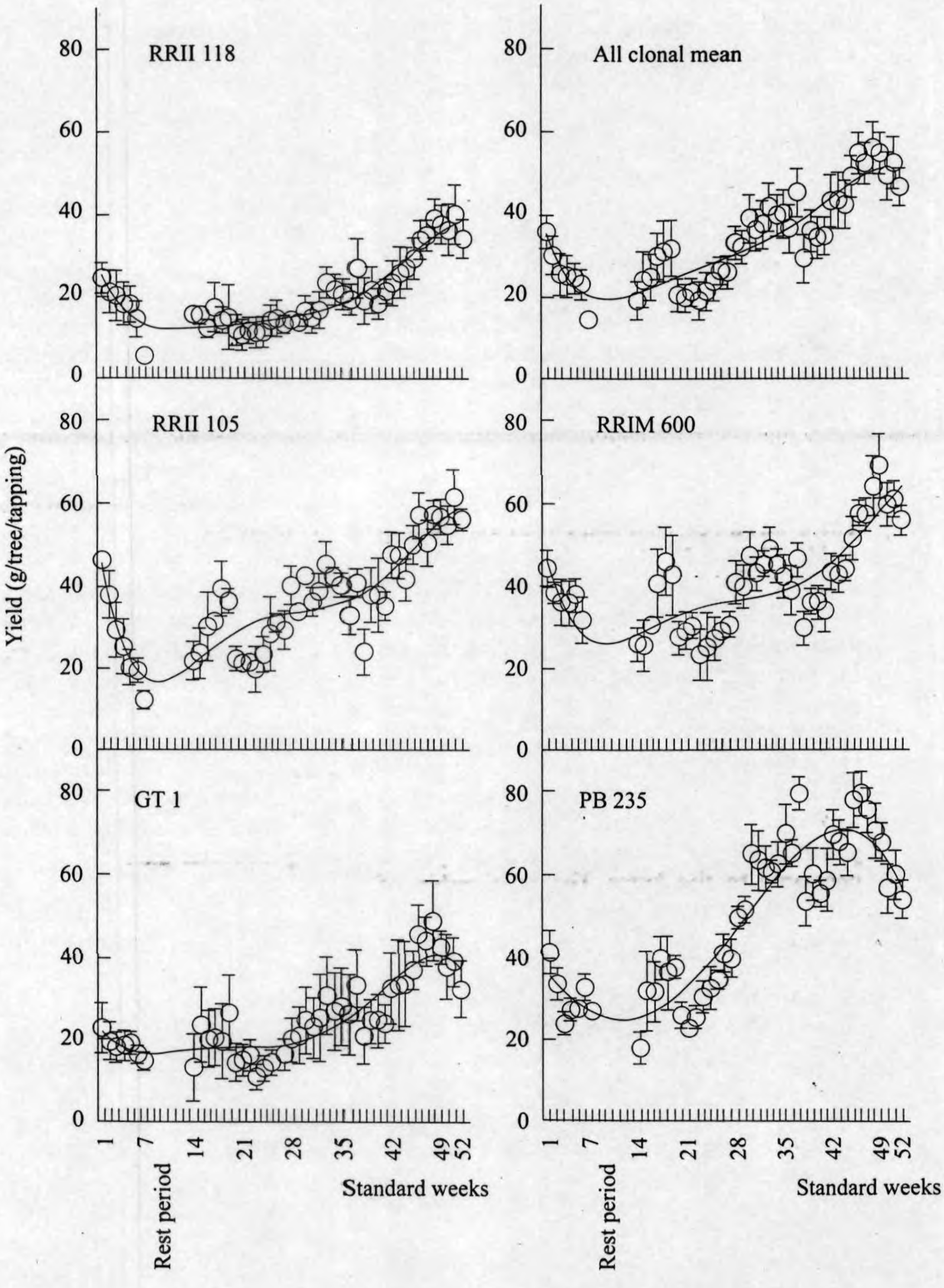

Figure 1. Dry rubber yield (g/tree/tapping) trend of different clones in different weeks in Hevea *grown n a non-traditional area of rubber cultivation. Rest period (from 8th to 13th week) means that there was no harvesting of latex during this duration. Vertical bars represent standard deviation (n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

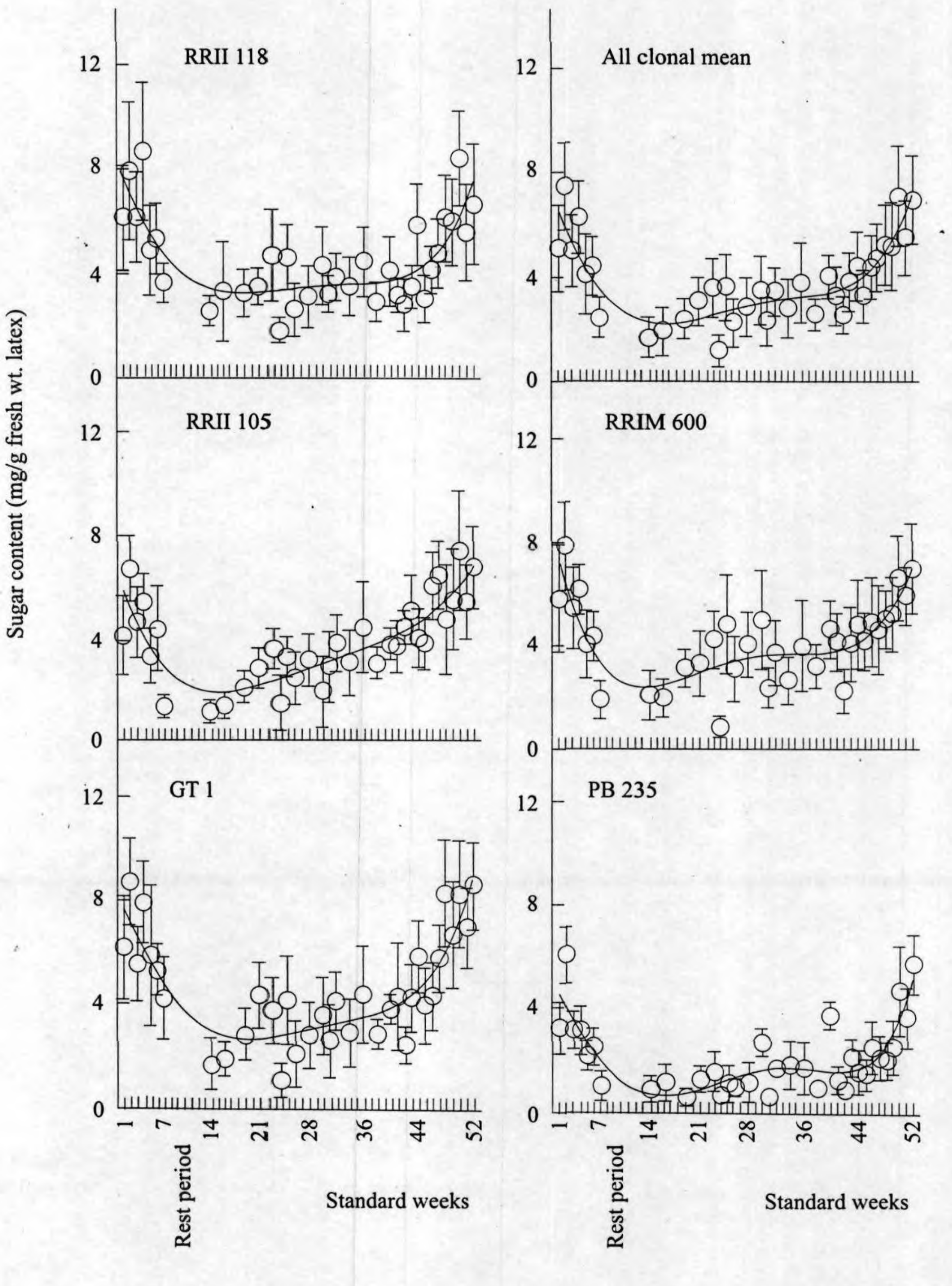

Figure 2. Trend of sugar content (mg/g fresh wt. latex) in latex of different clones in different weeks in Heve
grown in a non-traditional area of rubber cultivation. All the other conditions are same as in Figure 1.

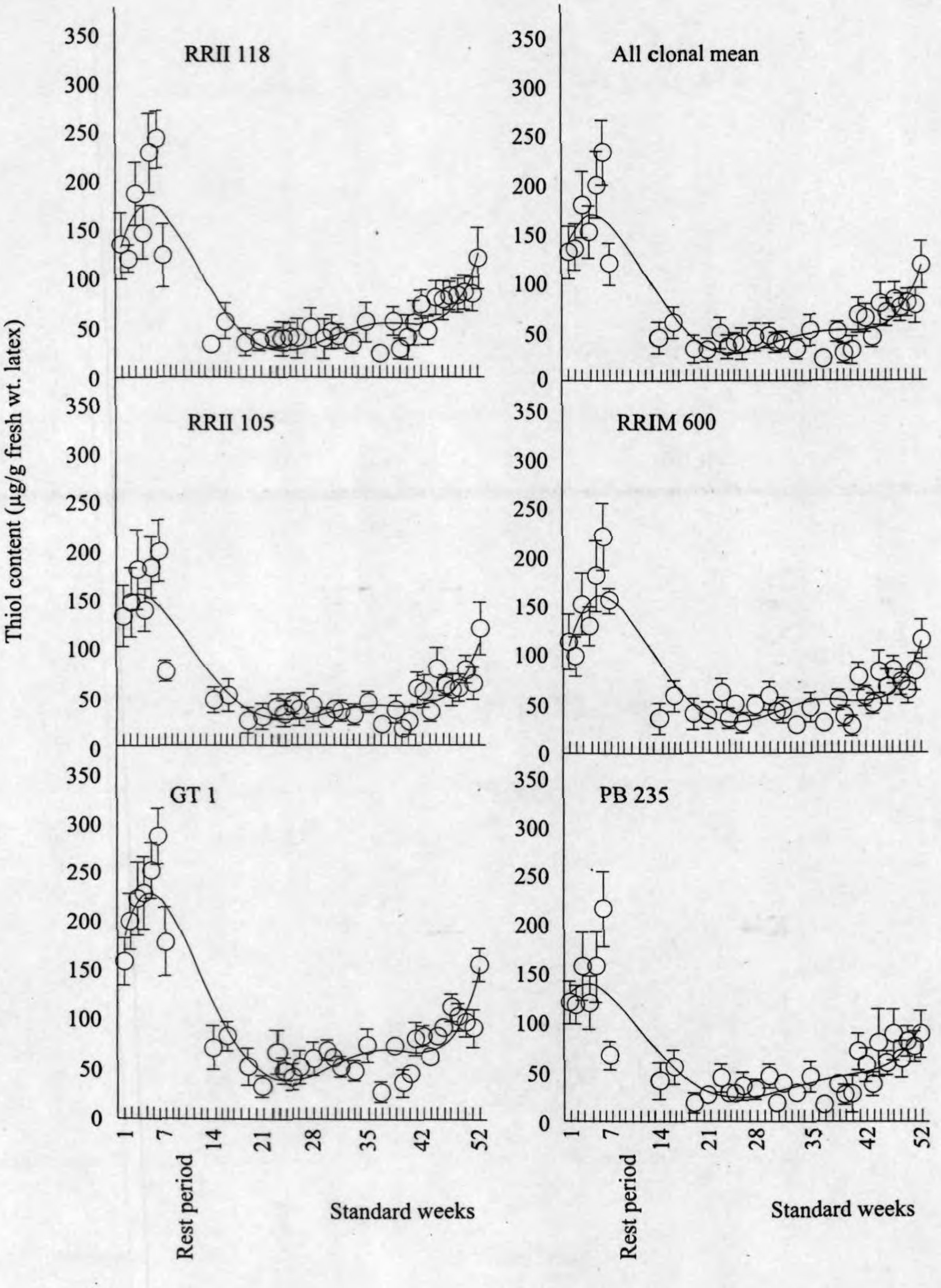

Figure 3. Trend of thiol content (µg/g fresh wt. latex) of latex in different clones in different weeks in Hevea *grown in a non-traditional area of rubber cultivation. All the other conditions are same as in* Figure 1.

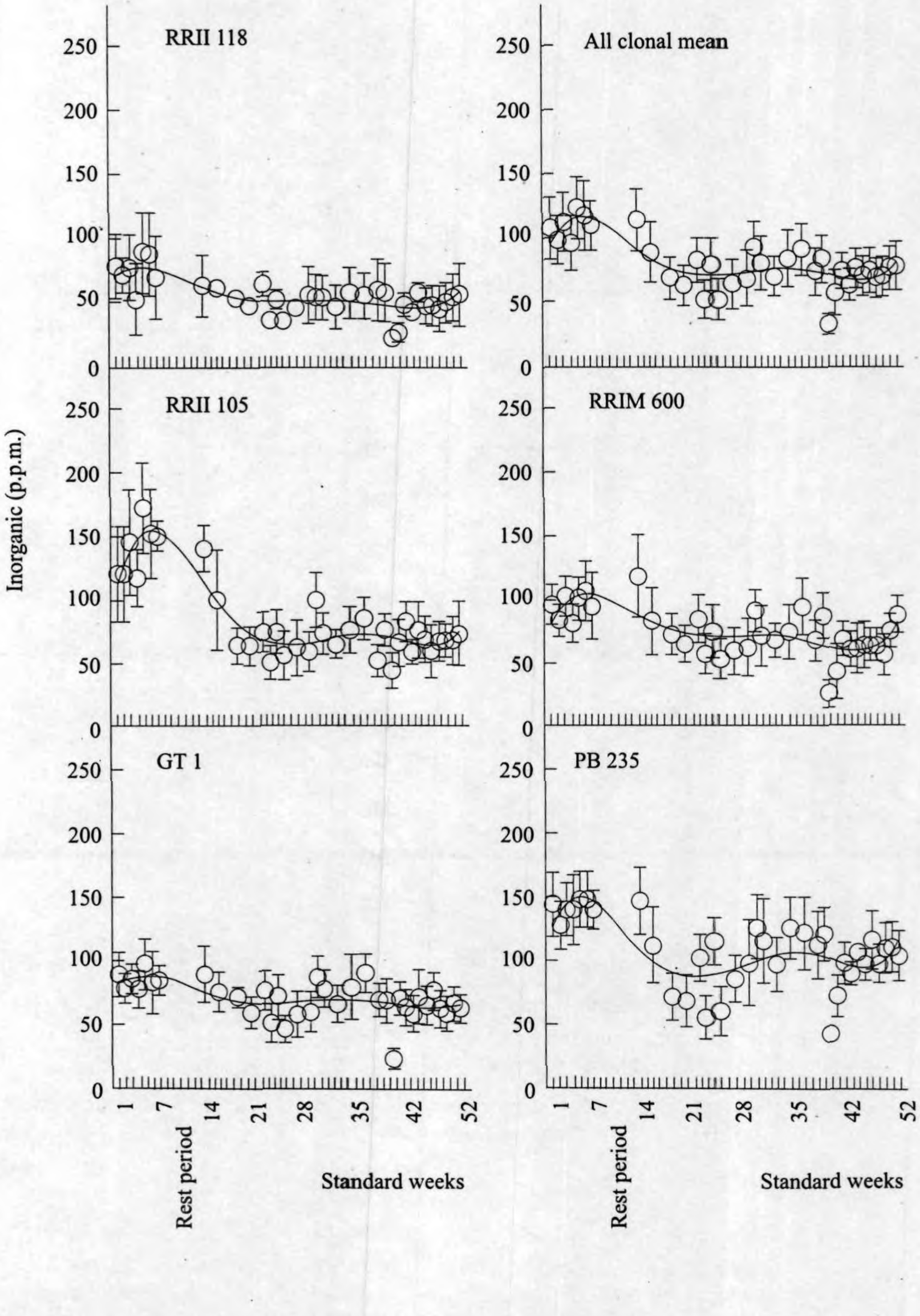

Figure 4. Trend of inorganic phosphorus content (p.p.m.) in latex of different clones in different weeks in Hevea *grown in a non-traditional area of rubber cultivation. All the other conditions are same as in* Figure 1.

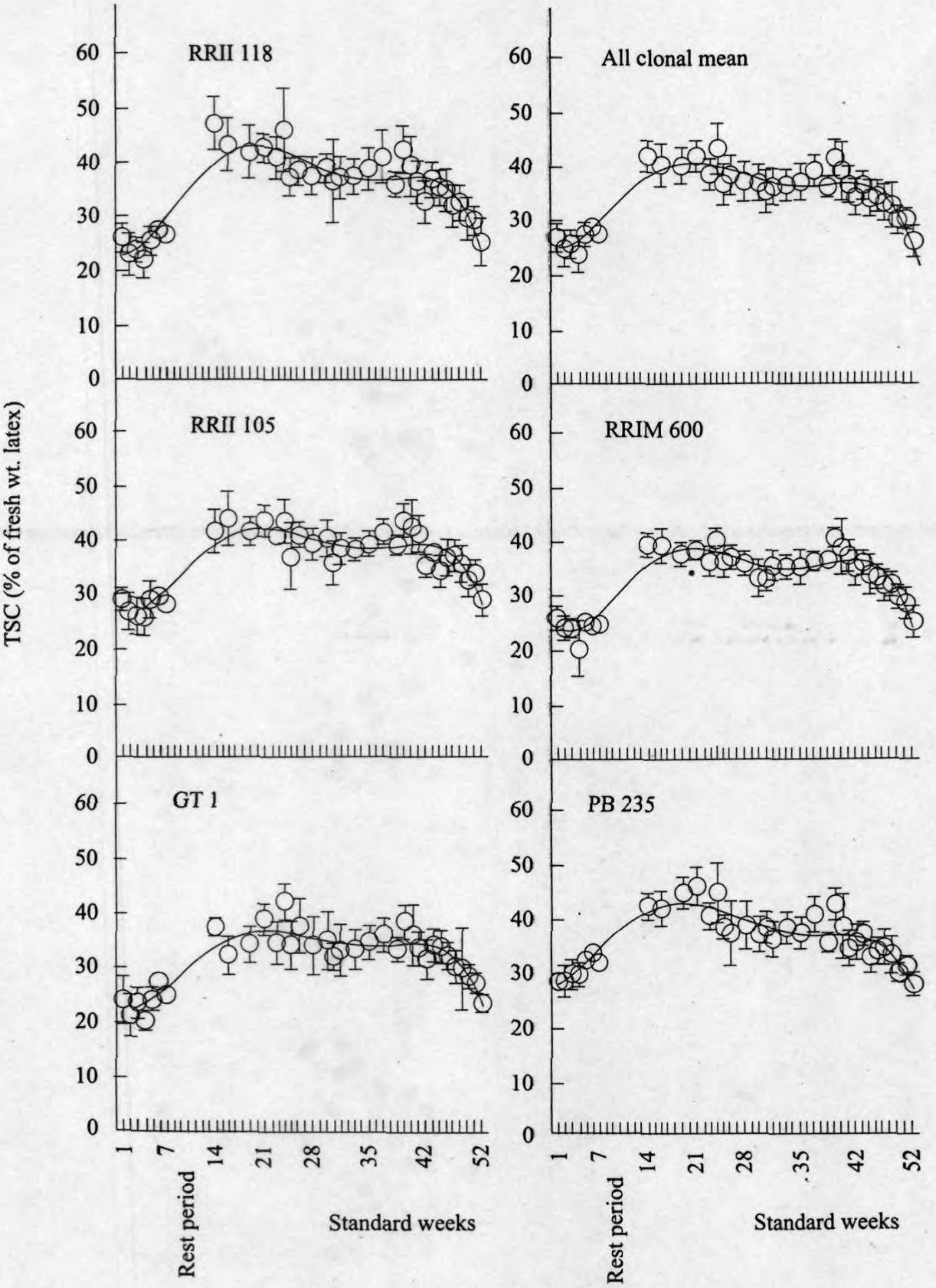

Figure 5. Trend of total solid content [TSC (% of fresh wt. latex)] of latex in different clones in different weeks in Hevea *grown in a non-traditional area of rubber cultivation. All the other conditions are same as in* Figure 1.

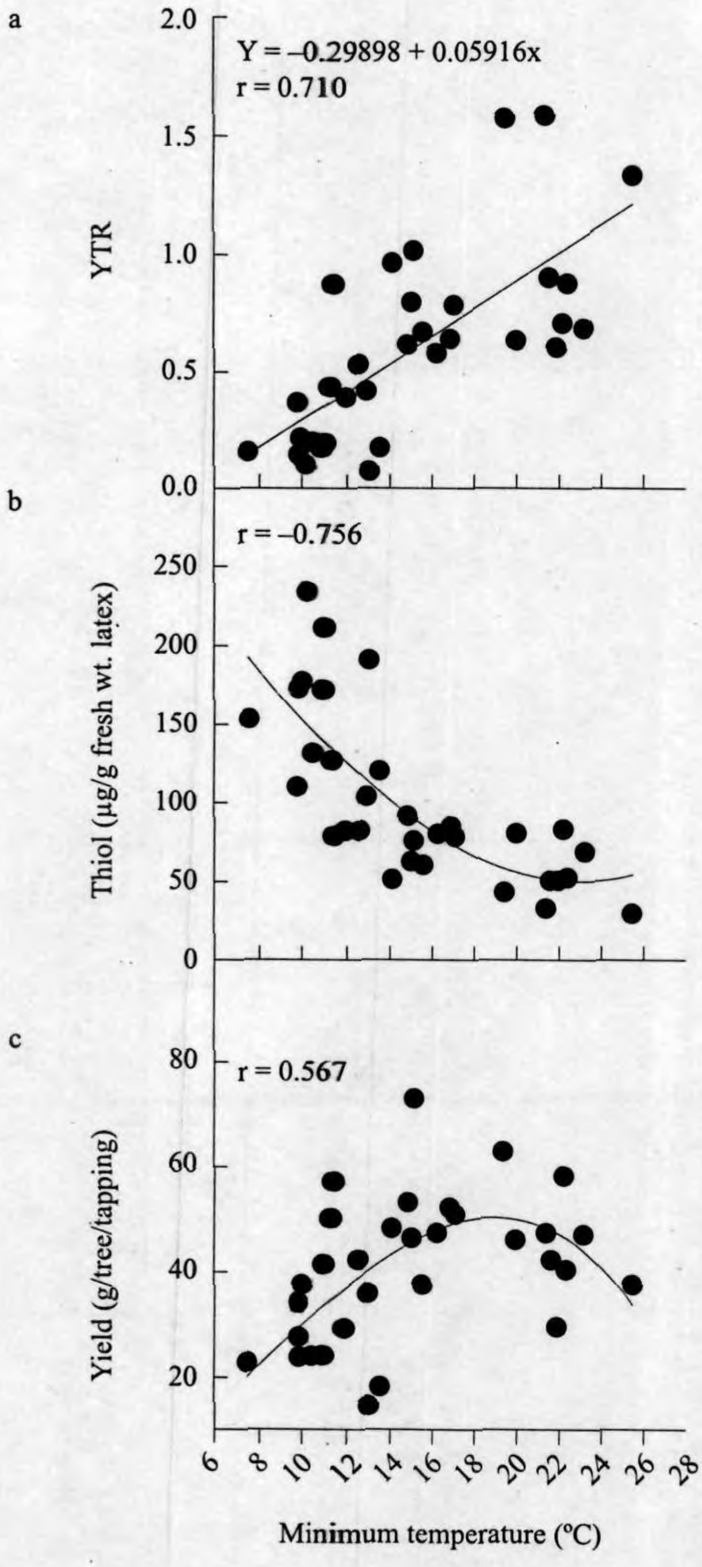

Figure 6. Effect of minimum temperature on YTR (a), thiol (b) and dry rubber yield (c) in Hevea *grown in a non-traditional area of rubber cultivation. [YTR (ratio of yield to thiol), thiol (µg/g fresh wt. latex), yield (g/tree/tapping)].*

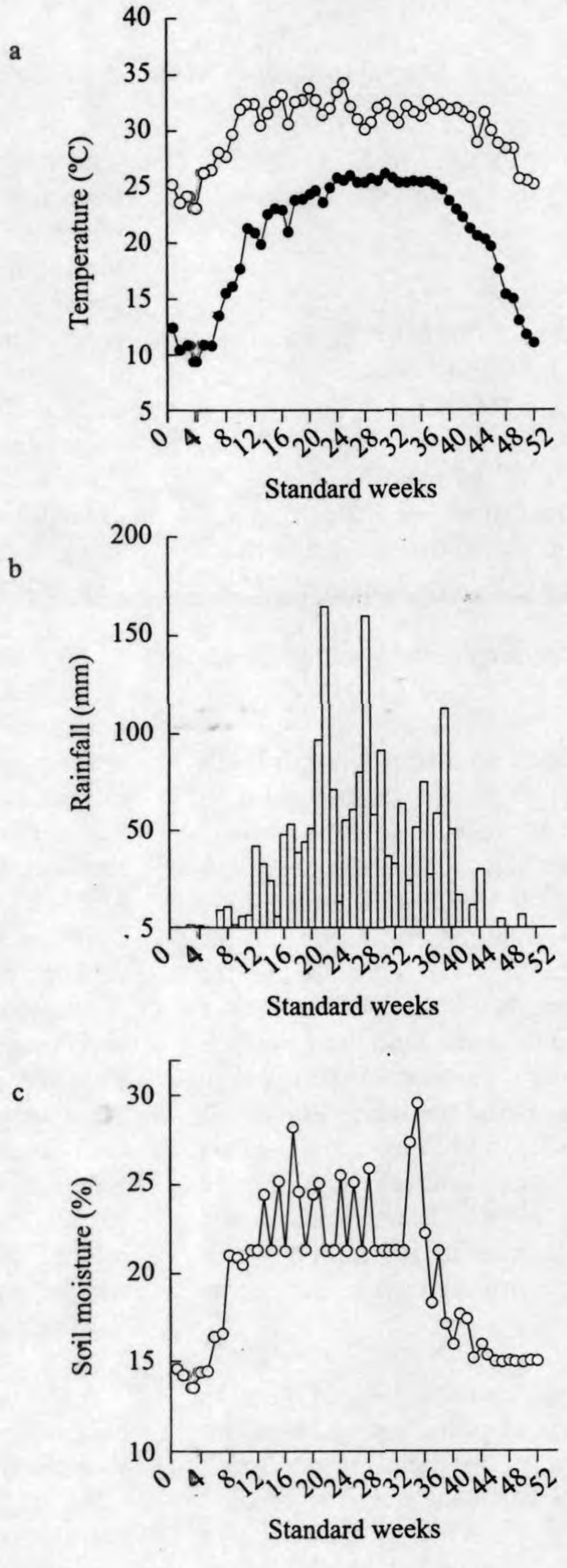

Figure 7. Climatic details [(a: Temperature (°C); b: Rainfall (mm); c: Soil moisture (%)] of the location presented as the weekly average of three years covering the experiment duration.

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

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

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[9,20]. 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 photosynthesis[21]. Path analysis (*Table 3*) revealed that both T_{max} and Pi have major direct effects on latex sugar content. T_{max} showed negative correlation with sugar which could be due to the increase in respiration which reduced sugar concentration with increase in temperature[22].

Leaves of trees contain 95% of thiol as glutathione[23] and thiol plays a very important role in various ways in plant metabolism[24]. 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 report[9], similar relationship was also reported[20]. 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 tempe ture-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 ATPs[24] and its synthesis is very tightly controlled[27]. Positive correlation between thiol and Pi supports the theory that low temperature triggered energy utilisation for thiol synthesis[28]. Pi showed significant negative correlation 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 Pi[29] where Pi acts 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

TABLE 1. CORRELATION COEFFICIENT MATRIX OF DIFFERENT PARAMETERS

Parameters	Sugar	Thiol	Inorg. P	TSC	T_{max}	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

*, ** Significant at 1% and 5% level, respectively.

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.

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

Parameters	Inorg. P	TSC	T_{max}	T_{min}	SM	Total r
Inorg. P	<u>0.2582</u>	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	<u>0.2623</u>	−0.3326	0.0844	−0.499*
T_{min}	−0.1045	−0.4304	0.2063	<u>−0.4231</u>	0.1093	−0.642*
SM	−0.0476	−0.3427	0.1482	−0.3094	<u>0.1494</u>	−0.402*

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

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 $x = T_{min}$.

<u>Clones</u>	<u>Equations</u>	
GT 1	$y = -0.1711 + 0.0327x$ (r = 0.711, p< 0.01)	...
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 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 yielding 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 non-optimal environment.

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