

Ontogenetic variations in flush development are indicative of low temperature tolerance in *Hevea brasiliensis* clones

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Abstract. Para rubber (*Hevea brasiliensis*) trees are naturally adapted to the Amazonian tropical climate. In India rubber trees are traditionally cultivated in the warm humid tropics of the south. Northeast India is a non-traditional area for rubber cultivation. A major limiting factor on tree growth in the northeast region is stress due to low temperature. Being a deciduous tree, rubber trees exhibit annual natural defoliation prior to the winter season, and ensuing new leaf growth usually coincides with the low temperature period. Flushing behaviour of trees during this period provides an opportunity to assess their winter hardiness. A study was carried out on five clones, RRIM 600, SCATC 93/114, GT 1, PB 5/51 and Haiken 1, to evaluate phenological behaviour of leaf growth during the period of low temperature stress. Trees were monitored for expansion of leaf area, internode length, petiole length and development of chlorophyll. Wide variation was observed among these clones for all the traits. SCATC 93/114 was better adapted for low temperature stress as this clone was found to have faster expansion of leaf area and better chlorophyll development, followed by Haiken 1. PB 5/51 was found to show poor performance during low temperature. Haiken 1 and PB 5/51 also exhibited better relative growth rate during winter months confirming their low temperature tolerance. Ontogenetic variations in leaf development are good indicators of assessing inherent cold tolerance in *Hevea* clones.

Keywords: Para rubber, *Hevea brasiliensis*, low temperature stress, growth rate.

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Introduction

The Para rubber tree, *Hevea brasiliensis* Muell. Arg., is a tropical tree native to Amazon rain forests. More than 99% of the world's natural rubber is obtained from rubber tree latex (Jacob et al. 1993). *Hevea* rubber alone accounts for about 40% of the total global rubber consumption which includes both natural and synthetic rubber. Global dependence of natural rubber is likely to increase further because of the fast shrinking resources of non-renewable energy sector, the petroleum industry, which source inputs for the synthetic rubber industry. The rubber tree is well adapted to humid tropics between 10° S to 10° N latitudes. Within the rubber tree plantation industry, this latitudinal belt is known as the traditional rubber growing region. In India, the traditional rubber belt encompasses the southern tip of the peninsula, where rubber has been grown on a plantation scale for over a century. Because of shrinking availability of cultivable land in traditional tracts, rubber cultivation in India has been extended to areas of diverse agroclimatic zones where near similar weather conditions prevail (Krishnakumar & Meenattoor 2000).

Compared to other crops, rubber is a relatively new introduction to India, having been brought into cultivation in the early 1900s. Study of adaptation responses of rubber clones thus bears extreme importance in this country. Adaptation studies generally involve two aspects, a) assessment of biological response including growth analyses and b) understanding the nature of adaptation for the formulation of selection strategies (Shorter et al. 1991). Rubber trees are deciduous, and the annual leaf fall is called 'wintering'. The new leaves appear immediately after leaf fall in a lush growth pattern. Young leaflets appearing after budburst are thin, glossy and copper brown, with high respiration and no photosynthesis (Leiberei et al. 1996, Miguel et al. 2007). Subsequently leaves develop a greenish appearance, turning completely dark green by 6-7 weeks after

emergence. Based on morphology, leaf development is divided into four stages, A, B, C and D (Dijkman 1951). Stage B is further subdivided into B1 and B2 (Hallé & Martin 1968). Physiologically, stage A, B and C leaves are completely sink leaves with no net positive energy balance (Leiberei et al. 1996) and stage D leaves are source leaves, which possess physiological and structural parameters of mature leaves (Leiberei 2007). Sink leaves are devoid of lignin and are susceptible to different biotic and abiotic stress factors. The sink leaf stage is the period of rapid cell elongation and multiplication.

Tripura in northeast India is an important non-traditional rubber-growing region where extensive rubber cultivation is being promoted. Rubber trees are naturally adapted to warm humid weather and low temperature in Tripura during the winter season is considered to be a major limiting factor for cultivation. In Tripura, leaf senescence starts during late November and extends up to early February, immediately followed by new leaf emergence (Vinod et al. 1996). This flushing generally coincides with low temperature spells. Sharp declines in ambient temperature below 15°C are very common during this period, forcing plants to support cell division only for aiding tissue differentiation but not rapid cell multiplication and elongation that should be happening normally under refoilation process (Zongdao & Xuekin 1983). Hence, low temperature tolerance is a highly desirable and a prime attribute to be studied among clones recommended for cultivation in this region. Furthermore, low temperature stress induces a carried forward lowering of rubber yield during May – September period in the ensuing year and those clones which are low temperature tolerant can put forth better growth during the winter period and yield well in the subsequent season (Priyadarshan et al. 2000). The present investigation was formulated to study the meristematic growth response of different clones exposed to low temperature, in order to assess the adaptability of these

clones to northeast India.

Materials and methods

Plant materials

Five oriental Para rubber tree clones, RRIM 600, PB 5/51, SCATC 93/114, Haiken 1 and GT 1 from a stress tolerance evaluation experiment planted on the research farm of the Rubber Research Institute of India, at Taranagar, Tripura (23° 53' N; 91° 15' E; 30m MSL) were used in this study. Five trees per genotype of the same age and height were selected. Trees were bud grafted clones of the respective clones developed on assorted *Hevea* rootstocks. Three healthy branches from each tree at similar levels above ground (~400 cm) were tagged for observations. Data were collected from flushes of similar morphology and age. All the trees were subjected to similar agonomic management.

Climatology

Climatologically, the northeast India (23-25° N and 90-95° E) has tropical, sub-tropical and temperate climate (at higher altitudes). Tropical weather is predominant with moderate temperature and high humidity. Tripura offers a representative tropical environment of the northeast India. Daily mean temperature ranges between 21.4°C to 32.6°C during summer and between 11.7°C and 26.3°C during winter months. Rains are common during southwest monsoon (June - September) and may extend to October. Annual average rainfall is 1970 mm received in about 112 rainy days. Average daily evapotranspiration is 3.4 mm. Low temperature during November to January, brief moisture stress during March, tornados, hailstorms and tropical storms during pre-monsoon (April - June) are the major climatic constraints for rubber cultivation.

Field measurements

Traits associated directly with rapid cell multiplication and cell elongation like leaf length (LL), leaf breadth (LB), petiole length (PL), and internode length (IL) were measured daily in the morning at 9.00 AM to 11.00 AM for two seasons of particularly low temperature, the 5th and 7th year after planting. The buds were selected in such a way that they appeared to be of the same size and the stage of growth. Measurements were initiated after bud burst, immediately after the appearance of leaf primordia, on each selected branch, from second week of February until complete expansion of leaves. Observations covered the five leaf growth stages viz., A, B1, B2, C and D (Dijkman 1951, Hallé & Martin 1968). Extreme care was taken to measure the young leaves and stem without inflicting any mechanical damage. Six weeks were required for complete expansion of the flushes to fully grown leaves for all the clones. The minimum daily air temperature ranged from 11.8°C to 17.2°C during the study period, with four out of six weeks having minimum temperatures below 15°C.

Measurement of chlorophyll development

Leaf samples were collected from flushes of same growth phase from the same trees used for field measurements at weekly intervals covering all leaf growth stages. The fresh leaves were cut into small pieces (16 mm²) and weighed. Chlorophyll was extracted using 68% dimethyl sulphoxide (DMSO) as extractant as per Tait and Hick (2003). 20 mg fresh leaf pieces were placed in a tube containing 10 ml DMSO and incubated in dark for 24 hours. When chlorophyll was completely extracted, the leaf pieces turned translucent. Three ml of chlorophyll extract was transferred to a transparent cuvette and the absorption values at 645 nm and 663 nm were read in a UV - VIS spectrophotometer. Chlorophyll *a* and *b* content were estimated with the empirical equations

used by Wellburn (1994).

Data analyses

The leaf area (LA) of the young leaves was determined using the length \times breadth (LB) method, based on the ratio constant constructed for individual selected leaflets, whose actual area was determined using a portable leaf area meter (LI - 300). The proportionate growth increment per weekly interval was calculated. The data were subjected to analysis of variance for determining the components of variation in the flushing behaviour across clones. Spearman's rank correlations were computed between clones across leaf growth stages within seasons and between seasons for all the traits. To compare the growth performance of the clones during winter months (November - Februarz), relative growth rate during the period was estimated (Hoffman & Poorter 2002) for the selected trees from their girth data from 5th to 8th year of planting. The biomass of the trees was estimated using the girth-biomass equation (Dey et al. 1996). All data analyses were done under R statistical computing environment (Venables & Smith 2010).

Results

Commencement of leaf development stages

Significant genotype differences were ob-

served for commencement of various stages of leaf development after bud burst (Table 1). The genotype, SCATC 93/114 showed early commencement of leaf growth, rapidly crossing stages A, B1 and B2 within 25 days and completing leaf maturity by 31 days, faster than any other clones. Haiken 1 was the next to follow this genotype by completing leaf maturity within 33 days. However PB 5/51, the slowest developing genotype, took about 35 days to complete sink leaf stages, attaining full maturity of the leaves by 43 days.

Variation in leaf growth

Means of leaf traits at different growth stages for the five clones during two seasons are presented in Tables 2 and 3. All the traits varied significantly amongst clones in both seasons. During the first season, expansion of LA ceased the fifth week in RRIM 600, Haiken 1, SCATC 93/114 and GT1 while it continued till week six in PB 5/51. LA was greatest for RRIM 600 and lowest for PB 5/51. However, elongation of IL and PL continued till week six in all the clones except for RRIM 600 for IL. SCATC 93/114 was found to have the longest internodes while Haiken 1 was shortest. The PL was found to range between 9.80 cm (PB 5/51 and Haiken 1) and 16.20 cm (RRIM 600). Correspondingly, in the second season, significant genotype difference in LA expansion was observed for all 6 weeks, while IL and PL showed no significant variation beyond

Table 1 Mean number of days over two years from budburst to commencement of ontogenetic stages of leaf development in five clones

Clones	Leaf development stage				
	A*	B1	B2	C	D
RRIM 600	5.70	14.20	22.20	29.30	37.30
PB 5/51	7.00	15.80	24.00	34.80	43.00
SCATC 93/114	1.80	7.80	13.80	25.20	30.80
Haiken 1	4.30	12.20	19.30	27.80	33.30
GT 1	5.00	13.80	20.80	30.00	35.30
SE (d)	0.29**	0.68**	0.35**	0.73**	1.82**

Note: * Stage A was counted from the day when middle leaflet of a top young leaf was 5mm long; ** Significant at $p = 0.05$

Table 2 Mean weekly values of traits at leaf ontogenetic stages of five clones in the first season (5 years after planting)

Clones	Days after budburst					
	0-7	8-14	15-21	22-28	29-35	35-42
Leaf area (cm ²)						
RRIM 600	0.05	0.50	10.70	40.70	56.10	56.10
PB 5/51	0.02	0.11	1.30	12.50	29.00	30.90
SCATC 93/114	0.24	1.48	19.40	40.60	48.20	48.20
Haiken 1	0.13	0.77	14.90	36.10	41.60	41.60
GT 1	0.08	0.38	11.10	40.10	50.90	50.90
SE (d)	0.01**	0.05**	0.60**	3.31**	2.16**	1.43**
Internode length (cm)						
RRIM 600	0.55	0.95	2.12	2.51	2.72	2.72
PB 5/51	0.25	0.37	1.22	1.75	1.97	2.39
SCATC 93/114	0.82	1.70	3.23	3.80	3.85	3.99
Haiken 1	0.45	0.80	1.57	1.92	2.10	2.15
GT 1	0.42	0.77	1.99	2.62	3.10	3.20
SE (d)	0.03**	0.04**	0.20**	0.14**	0.14**	0.14**
Petiole length (cm)						
RRIM 600	1.03	2.52	11.70	15.77	16.12	16.20
PB 5/51	0.34	0.65	4.40	8.52	9.77	9.80
SCATC 93/114	3.01	5.27	12.77	13.60	13.90	14.00
Haiken 1	1.75	2.97	8.50	8.75	9.57	9.80
GT 1	1.00	2.15	10.27	12.55	13.87	14.00
SE (d)	0.04**	0.14**	0.24**	0.72**	0.81**	0.76**

Note: ** Significant at $p = 0.01$ at genotype level

the third week. LA was highest for GT 1 followed by RRIM 600, while PB 5/51 had the lowest. IL ranged from 1.45 cm (GT1) to 3.27 cm (SCATC 93/114), while PL ranged between 11.88 cm (RRIM 600) and 16.20 cm (Haiken 1).

To know whether the pattern of genotype behaviour was similar for all the weeks within as well as between seasons, rank correlations were computed. The results are presented in Table 4. It was found that genotype performance for expansion of LA between seasons was more consistent for all weeks than was IL & IP.

The growth increment for LA, IL and PL are depicted in Fig. 1. The highest rate of expansion was found up to 28 days after budburst for all the traits in general. However, expan-

sion rate differed considerably among clones for different traits. SCATC 93/114 consistently exhibited fastest expansion rate in both seasons for all the traits. In contrast, PB 5/51 showed the slowest expansion rate with an expansion pattern spanning more than six weeks. GT 1, RRIM 600 and Haiken 1 exhibited intermediate expansion pattern.

Chlorophyll development

The pattern of chlorophyll build-up in young growing leaves is given in Fig. 2. The content of chlorophyll *a*, *b*, and total increased as the leaves grew in all the clones. Highest rate of increase of chlorophyll content occurred between 21 to 35 days after budburst. Significant genotypic variation was observed in chloro-

Table 3 Mean weekly values of traits at leaf ontogenetic stages of five clones in the second season (7 years after planting)

Genotype	Days after budburst					
	0-7	8-14	15-21	22-28	29-35	35-42
Leaf area (cm ²)						
RRIM 600	0.07	0.48	11.14	43.90	58.31	60.01
PB 5/51	0.07	0.20	1.92	17.25	39.79	41.49
SCATC 93/114	0.18	1.11	13.76	34.31	44.59	45.42
Haiken 1	0.05	0.22	4.87	30.04	55.27	58.22
GT 1	0.04	0.38	8.30	42.07	63.92	65.34
SE (d)	0.05**	0.03**	0.43**	7.30**	12.74*	13.69*
Internode length (cm)						
RRIM 600	0.33	0.53	1.42	2.02	2.12	2.12
PB 5/51	0.15	0.33	0.83	1.23	2.07	2.48
SCATC 93/114	0.67	1.37	2.78	3.03	3.18	3.27
Haiken 1	0.32	0.52	1.33	2.72	2.85	2.90
GT 1	0.25	0.43	0.82	1.25	1.40	1.45
SE (d)	0.01**	2.88*	4.85*	ns	ns	ns
Petiole length (cm)						
RRIM 600	1.32	2.75	8.00	11.67	11.82	11.88
PB 5/51	0.57	1.52	6.28	11.93	14.33	15.47
SCATC 93/114	3.47	6.45	11.07	13.65	13.95	14.07
Haiken 1	2.12	3.02	9.63	15.05	15.60	16.20
GT 1	2.52	4.18	11.38	15.68	16.00	16.12
SE (d)	0.59**	1.09*	1.93*	ns	ns	ns

Note: ns - non-significant, *- significant at $p = 0.05$, ** - significant at $p = 0.01$, respectively at genotype level

phyll development. There was higher content of both chlorophylls *a* and *b* with faster build-up in SCATC 93/114, followed by Haiken 1 and RRIM 600. PB 5/51 had poor chlorophyll build-up. A steady pattern of chlorophyll *a/b* ratio increase was observed only in SCATC 93/114, while RRIM 600 had an initial fall in the ratio which no other clones exhibited. A significant increase in chlorophyll *a* content was seen in PB 5/51 after day 28 following budburst, resulting in a sudden increase in the *a/b* ratio.

Relative growth rate

The estimated mean relative growth rates during

winter months of the clones for four seasons are depicted in Fig. 3. It was found that, among the clones, the highest RGR was exhibited by genotype SCATC 93/114, followed by Haiken 1 and RRIM 600. The lowest RGR was recorded by PB 5/51.

Discussion

Tree growth in general is attributed to the production of cells per unit volume, and their elongation potential. Cell multiplication and elongation are controlled genetically by varying mineral and hormonal allocations (Gomez & Hamzah 1980). Development of leaves in

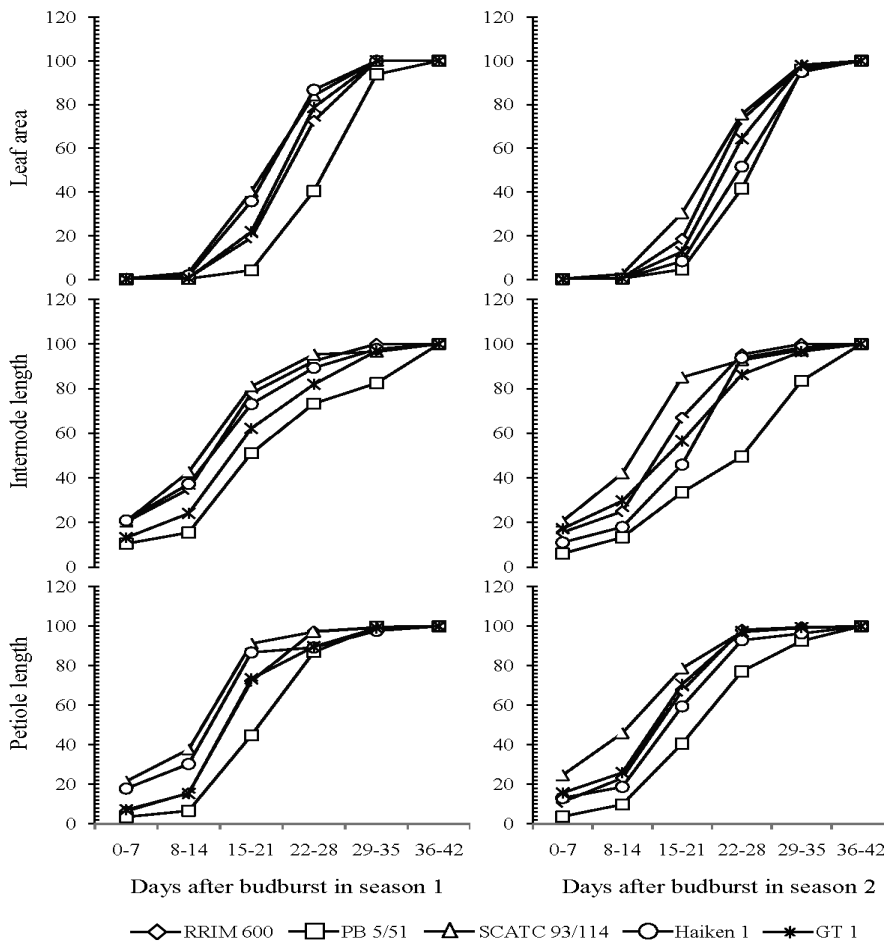


Figure 1 Growth increment (%) of five clones for leaf area, internode length and petiole length during seasons 1 and 2

early stages of growth consists predominantly of active cell division, differentiation and development of chloroplast complexes for building the photosynthetic apparatus. These activities are highly energy demanding and they are distinct in different leaf development stages (Miguel et al. 2007). Variation in the development of a genotype which allows it to effectively spend energy towards growth and establishment under low temperature stress can be considered as tolerant to low temperature. Another genotype with poor energy conservation

ability during low temperature is non-adapted to winter even if it compensates for the decrement in growth and development during the non-winter period, while a third genotype which shows both the above characters, can be considered more widely adapted.

In the present investigation, development of LA and chlorophyll during the juvenile stage was the most important trait observed because this development ultimately determined the size of the photosynthetic apparatus of the tree. Photosynthesis and respiration together

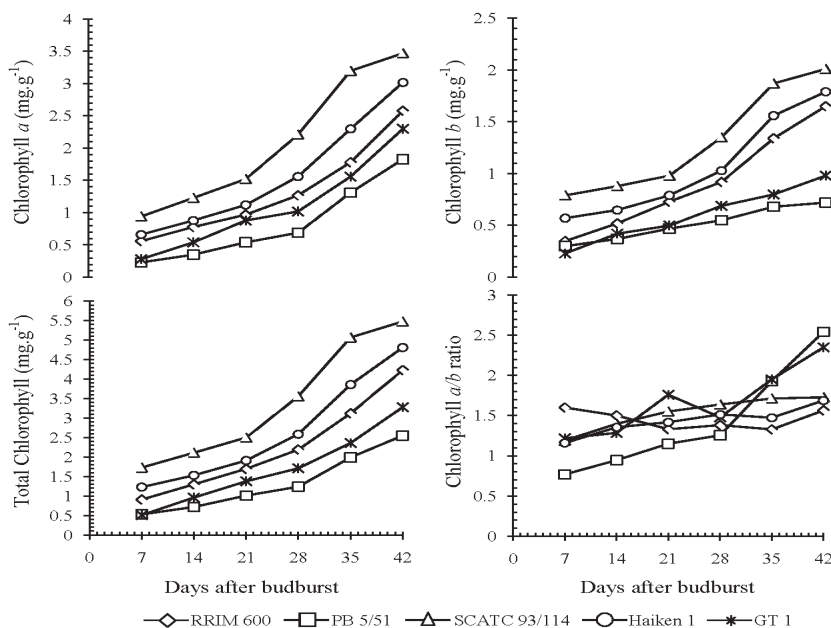


Figure 2 Contents of chlorophyll a, b, total and a/b ratio during leaf ontogenetic process in five clones

govern the dry matter increase in rubber tree leaves at this stage and is directly related to the balance between CO₂ assimilation, and its release. A high rate of respiration at stages A, B and C of leaf development therefore significantly affects CO₂ balance in rubber tree. Increased respiration indicates a high metabolic activity, by which energy is released to synthesise structural compounds and chlorophyll (growth respiration), as well as to the maintenance (maintenance respiration) of formed compounds (Bergonci 1981).

On the other hand, IL and PL determined the secondary aspects of leaf aestivation and light interception. Furthermore, IL and PL traits have a shorter maturity period than leaf area, as observed from the relation of weeks within seasons, where significant association was found only between adjacent weeks, as well as from the non-significant variation observed in the later weeks of the second season. In the present situation, the first four week period of active

cell division coincided with temperatures below 15°C, exposing trees to low temperature stress. This period was very crucial because this was the most susceptible period for stress injury of young leaves (Leiberei 2007). Therefore, it can be rationalised that the extent of expansion time in LA, and chlorophyll build-up can substantially contribute to the ability of the genotype to withstand the low temperature. Shorter the time for sink leaf stage better will be the low temperature tolerance.

Results of the present study emphatically point out that faster reestablishment of canopy structure and quick development of the photosynthetic apparatus observed in SCATC 93/114 was a key observable feature resulting in higher RGR values of this genotype due to its better adaptability to winter. SCATC 93/114, a high yielding low temperature tolerant genotype developed in China (Huasun & Shaofu 1990) did show a significant positive growth rate for all traits studied. It is

Table 4 Spearman's rank correlations between weeks for three traits within first (upper diagonal) and second (lower diagonal) season, and between the seasons (diagonal elements)

	Traits	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Week 1	LA	0.871*	0.954**	0.913**	0.454	0.254	0.236
	IL	0.956**	0.989**	0.809*	0.586	0.589	0.599
	PL	0.814*	0.956**	0.431	0.320	0.200	0.201
Week 2	LA	0.988**	0.853*	0.934**	0.445	0.350	0.356
	IL	0.987**	0.945**	0.822*	0.619	0.639	0.666
	PL	0.994**	0.830*	0.609	0.404	0.385	0.375
Week 3	LA	0.867*	0.799*	0.766*	0.765*	0.584	0.564
	IL	0.932**	0.943**	0.600	0.953**	0.925**	0.942**
	PL	0.885*	0.845*	0.787*	0.916**	0.911**	0.902**
Week 4	LA	0.697	0.623	0.987**	0.542	0.959**	0.957**
	IL	0.654	0.677	0.845*	-0.095	0.983**	0.994**
	PL	-0.005	0.103	-0.008	-0.544	0.967**	0.989**
Week 5	LA	0.313	0.266	0.649	0.846*	0.713*	0.999**
	IL	0.622	0.645	0.805*	0.997**	0.003	0.990**
	PL	-0.030	-0.116	-0.052	0.989**	-0.605	0.997**
Week 6	LA	0.281	0.243	0.629	0.789*	0.999**	0.679
	IL	0.645	0.653	0.823*	0.997**	0.997**	0.054
	PL	-0.045	-0.154	-0.112	0.983**	0.991**	-0.534

Note: LA – Leaf area (cm²) IL – Internode length (cm) PL – Petiole length (cm); *, ** significant at $p = 0.05$ and $p = 0.01$ respectively at weekly level

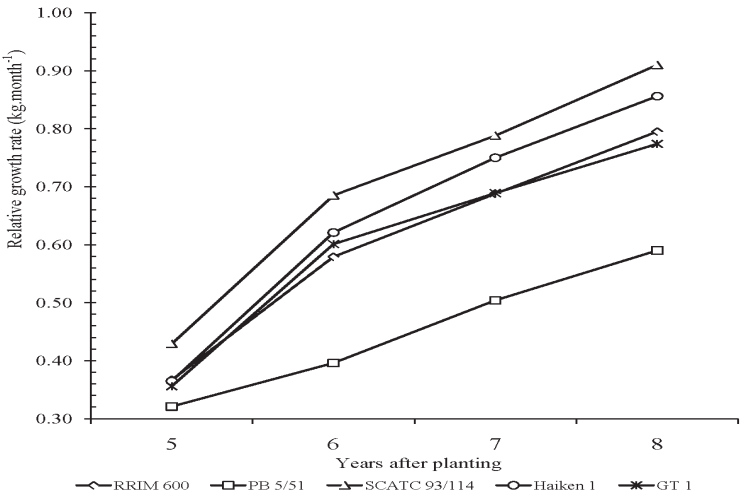


Figure 3 Relative growth rate (kg.month⁻¹) of five clones during winter months

worth mentioning that this genotype has better annual girth increment in northeast India. Another genotype worth mentioning here is Haiken 1, which showed moderate leaf expan-

sion and faster chlorophyll development, and a high growth rate during winter. Haiken 1 is a candidate cold tolerant clone from China, specifically adapted to low temperature stress

(Priyadarshan et al. 1998) and widely planted in cooler locations of China. Further, SCATC 93/114 and Haiken 1 showed the highest chlorophyll build up between stages C and D, commencing from the late B2 stage. A similar response for these clones was reported earlier by Miguel et al. (2007). Clones RRIM 600 and GT 1, produce long petioles, good leaf area and internode length, by the end of cooler period, have a greater ability to harvest more sunlight during non-winter period, enabling them to be adapted to normal weather with moderate annual growth rate. Growth adaptability for girth increment under northeast conditions of GT 1 and RRIM 600 was reported earlier by Rajeswari et al. (1991). These clones probably have better compensation mechanism for making good of lower response in growth by reducing the energy expense during actual stress period and utilising it more efficiently towards growth under favourable conditions. Though not better than established cold tolerant clones, RRIM 600 and GT1 rank below SCATC 93/114 and Haiken 1 in genotype evaluation trials in the northeast (Priyadarshan et al. 2005). In contrary, PB 5/51, has a longer expansion span, poor chlorophyll build-up and smaller leaves. This genotype may not be ideal for the northeast region, as it was also reported to be poor in early establishment, growth and yield (Vinod et al. 1996).

Conclusion

The present study provided an opportunity to assess genotype responses to low temperature, by measuring simple traits like leaf expansion and chlorophyll development during the actual stress period. It has been established that combinations of these parameters define net photosynthesis, water use efficiency and carboxylation efficiency of the clones (Miguel et al. 2007). Inclusion of two already established cold tolerant clones and long term growth data of many clones in this region could help us in

determining the cold tolerance of these clones. It is now clear that a faster transition from sink to source leaf stage in growing flushes is a key response to mitigate low temperature stress. These faster transition clones will have a fully functional photosynthetic apparatus even before the end of the actual stress period, enabling them to replenish food reserves that were expended during the 'wintering' period supporting active cell growth, differentiation and cell hardening. Therefore it can be concluded that the rapid development of leaves from source to sink is a good indicator for assessing inherent cold tolerance in *Hevea* clones.

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