

Evaluation of Brazilian wild *Hevea* germplasm for cold tolerance: genetic variability in the early mature growth

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Abstract *Hevea brasiliensis* is a commercially cultivated species for its natural rubber (NR) latex in South East Asian countries. To meet the ever-increasing demand, NR cultivation has been extended to non-traditional regions due to the limited scope of further expansion in traditional rubber-growing areas in India. These areas are often confronted with various abiotic stresses, especially high and low temperatures, which cause reduction in plant growth, thereby increasing its uneconomical immaturity period. Eighteen wild *Hevea* accessions along with two modern clones RRIM 203 and PB 235 and two check clones RRIM 600 and Haiken 1 were evaluated in the early mature growth phase. The site was at Nagrakata, West Bengal, the sub-Himalayan cold-prone region of India. In *Hevea*, crop production is governed by two major factors, growth-vigor and production capacity. Growth-vigor is of special importance because the production of rubber is a process linked with the early growth of the plant, which results in early tappability and early economic gains. The genotypes exhibited highly significant clonal differences ($P = 0.05$) for all the growth traits. Tappability percentage in the seventh year, ranged from 0.33 % (AC 3074, AC 3075, AC 3293) to 89.67 % (RO 2727). The most vigorously growing accession (RO 2727) reached tappable girth early in the seventh year when

the girth of plant ranged from 22.38 cm (AC 3293) to 53.12 cm (RO 2727). The general mean was 43.32 cm, and the similar growth trend was exhibited by these accessions in the tenth year also. Annual girth increment (cm a^{-1}) over 3 years ranged from 1.81 cm (AC 3075) to 6.80 cm (RO 2727). The mean winter girth increment (cm a^{-1}) over 4 years ranged from 0.13 cm (AC 3075) to 0.96 cm (RO 2727) as compared to the check clone RRIM 600 (1.11 cm) and Haiken 1 (1.10 cm). Wide differences between the phenotypic coefficient of variation (50.29) and genotypic coefficient of variation (24.82) were observed for winter girth increment. Girth in the tenth year recorded the highest heritability (87 %). Girth was significantly correlated with the other growth traits. The top 30 % of the potential accessions showing high growth vigour and early tappability under cold stress were identified. These ecotypes/selections have high potential value for the development of cold-tolerant clones for these regions and also in broadening the genetic base of the present-day cultivated rubber.

Keywords Cold tolerance · Genetic variability · Growth vigour · *Hevea brasiliensis* · Natural rubber · Wild accessions

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Introduction

The Para rubber tree (*Hevea brasiliensis* Muell. Arg.) is a strategic industrial crop cultivated mainly for its natural rubber (NR) latex in the Southeast Asian countries. Rubber is a perennial crop, and has a comparatively long gestation period. Girth is the most important parameter for growth evaluation and for opening the trees for harvesting latex (Paardekooper 1989). One of the important economic characters to be considered in the breeding and selection of

Hevea clones is vigour in terms of girth increase. Vigorous clones reach tappable girth early thus reducing the unecological immature period (Gan et al. 1991). Plant vigour during immaturity and yield precocity have been confirmed to be two important factors determining the overall early productivity of clones.

Tapping is a process of controlled wounding of the bark of the tree for harvesting latex rubber. At the beginning of the rubber culture in the Pacific, the trees were put into tapping more or less according to the individual ideas of the planters (Ridley 1897). More recently, a girth of 50 cm at 150 cm above the union for budded plants and a girth of 50 cm at 50 cm above ground level for seedlings were used in different rubber growing countries as criteria for selecting trees for tappability (Paardekooper 1989). Nevertheless, some are of the view that opening budded plants early, at 45 cm at 150 cm height from the union, reduces the immature period and also improves the profitability of estates (Ng et al. 1972). In commercial plantations, the girth of the trees is routinely measured starting at the fourth year, to determine when the trees may be put into tapping.

One of the most important aspects in plantation management is shortening the unproductive phase so that early return from investment is ensured. Since the early 1960s, various attempts have been made to reduce the period of immaturity. This includes the use of fertilizers and legume covers (Watson 1963). Split application of fertilizer and the use of increased rate and frequency of application lead to reduction in immaturity (Pushparajah et al. 1974). Reducing the immature phase is particularly important in non-traditional regions where the popularly grown cultivars can take one to 3 years longer period to reach tappable girth as compared to their counterparts in the traditional region (Reju et al. 2001). This is because the cultivation is extended against odds such as marginal situations, other agro-climatic features, and unskilled management. Early agro-management practices count a lot in the establishment, early growth, and uniformity of stand in the plantation from the beginning to the mature phase. Selection of the right type of planting materials plays a key role, where the genetically advanced planting material with desirable high genetic diversity upon introgression is known to accelerate growth vigour to reach tappable girth of trees earlier than the existing long approach.

In rubber-growing countries, the genetic advance gained in the early breeding phases seems to have slowed down in the more recent phases of breeding (Tan 1987) for which the narrow genetic base is considered to be one of the major reasons. In order to protect the depletion of genetic resources from their native region (Wycherley 1968), a huge collection of wild *Hevea* germplasm was gathered at its center of origin in the Amazon rainforests of Brazil, by the International Rubber Research and Development Board

during 1981 (Ong et al. 1983). The expedition area comprised three states in Brazil—Acre (AC), Rondonia (RO) and Mato Grosso (MT) and a share of these germplasm was distributed to member countries including India. Around 4548 accessions are being conserved in source nurseries in India, and are under different stages of evaluation for identification of desirable genes.

To meet the increasing global demand for natural rubber, rubber cultivation has been expanded to non-traditional regions in India, due to the limited scope of further expansion in traditional area. These areas are often confronted with various biotic and abiotic stresses, especially drought and temperature extremes. Cold stress in particular, is a serious threat for growth and development of *Hevea* plants and to the sustainability of crop yields in northeast India (Sethuraj et al. 1991; Rao et al. 1993). Various phenotypic symptoms in response to cold stress include poor germination of seeds, stunted growth, and in severe cases, leaves turning yellow. Growing tips dry up and further cold stress can lead to major crop losses.

However, acclimatization of *Hevea* to stress-prone areas (low temperature, higher altitude, wind, and diseases) in China giving profitable return (Fu and Xu 2005) has led to establishment of rubber cultivation in the or near to sub-Himalayan range of NE India, including Tripura, Assam, Meghalaya, and northern part of West Bengal. Since no information is available on wild germplasm in the sub-Himalayan region of West Bengal, the present study was undertaken in a cold-prone region to evaluate the growth performance of wild germplasm accessions in the early mature phase and to ascertain the extent of genetic variability for these traits in the population.

Materials and methods

The study was conducted at the Regional Experiment Station of the Rubber Research Institute of India, Nagrakata (Latitude 26°54'N, longitude 88°25'E and altitude 69 m MSL and annual rain fall 3300 mm) in the Jalpaiguri district of West Bengal, sub-Himalayan region of India, where in the peak winter period, the minimum winter temperature falls below 5 °C. A set of 18 wild *Hevea* accessions, two modern clones (PB 235 and RR II 203) along with two check clones (RRIM 600 and Haiken 1), were planted in a field trial in a randomized block design during 2000, with three replications. The spacing adopted was 4.9 × 4.9 m with five plants per plot, and the recommended cultural practices of Rubber Board were followed. Among the 18 wild germplasm accessions, five were from Acre, four from Mato Grosso, and nine from Rondonia provenance (Table 1). On the basis of weather variables, the entire year in this sub-Himalayan region can be broadly classified into two groups:

Table 1 Current status, country of origin and provenance wise distribution of *Hevea* accessions and clones

Accessions/clones	Subtotal	Country of origin/Provenance	Status
AC 3074, AC 3075, AC 3293, AC 3514, AC 3810	5	Brazil/Acre	Wild
MT 1020, MT 2229, MT 915, MT 900	4	Brazil/Mato Grosso	Wild
RO 2901, RO 2886, RO 2638, RO 2908, RO 2727, RO 2948, RO 3043, RO 3169, RO 3197.	9	Brazil/Rondonia	Wild
RRII 203*, PB 235**	2	*India, **Malaysia	Modern hybrid clones
RRIM 600*, Haiken 1**	2	*Malaysia, **China	*Hybrid and **primary check clones

- (1) Weather Regime I: Comprising the non-winter period, from April to September, mainly consisting of summer, monsoon, and post monsoon seasons in this area. In the non-cold season, temperature ranges from 23.1 to 31.5 °C and 77 % of the annual rainfall is received in this period.
- (2) Weather Regime II: Comprising the winter period, from October to March, and mainly consisting of cool and cold winter seasons only. In the cold season, temperature ranges from 5 to 28 °C, comparatively high sunshine hours, and the low (5 °C) temperature observed during December to January.

Data on girth (cm) of the plant was recorded at 125 cm height from the bud union. Growth during the winter cold stress period was taken as an indication of tolerance to cold. Winter growth was recorded as the girth increment (cm) of a plant during winter by computing the difference between pre- and post-winter girth, for four consecutive years, from the sixth year after planting. Pre-winter girth was recorded in the month of September (last week) continuously from 2006 to 2009, while post-winter girth was recorded in the first week of April every year from 2007 to 2010. The post-winter girth recording commenced with the annual girth recording every year. The average annual girth increment (cm) per year over 3 years was calculated using the annual girth data of the seventh and tenth years. Tappability regarded as 45 cm girth at a height of 125 cm from the bud union (McFadyean 1944; IRRDB 1984) was assessed as the percentage of the tree stand per plot in each accession/clone, in the seventh year to tenth year after planting, computed using the formula:

Tappability percentage =

$$\frac{\text{No. of trees attained a tappable girth of 45 cm in the plot}}{\text{Total No. of trees in the plot}} \times 100$$

By using absolute mean tappability percentage values from 2007 to 2010, a mean tappability percentage was estimated over 4 years and the accessions were ranked. The tappability percentage data values were converted using Arcsine transformation for statistical analysis. The data were subjected to analysis of variance (ANOVA) for

randomized block design and simple correlation among various growth characters were computed (Panse and Sukhatme 1989). Genetic components of variation and broad-sense heritability were estimated as per Singh and Choudhary (2007). The overall performance of all these accessions were assessed by the rank sum method (Kang 1988), using the traits tappability percentage; annual girth (cm) in the tenth year after planting; and mean annual girth increment (cm/year) over 3 years. Based on the mean value of a character, each accession was ranked, giving the highest rank to the best performer. Then the ranks across all the traits for each accession were pooled to give a rank sum. Hence the highest rank sum indicated the best performer (rank “1”).

Results

The climate of the northern region of West Bengal is sub-humid with an annual rainfall of 3300 mm and about 77 % of the annual rainfall is received between May and September. The maximum temperature rises as high as 31.5 °C during July and the minimum temperature is as low as 5 °C during December to January. From November to March, the temperature range between minimum and maximum is wide (12–15 °C) and the rainfall is less than 60 mm. In the present investigation, the growth pattern and behavior of different wild genotypes under these environmental conditions was examined. The genotypes exhibited highly significant clonal differences ($P = 0.01$) for all five quantitative traits studied except for winter-girth increment which was significant at $P = 0.05$ level (Table 2). The range and population mean values in comparison with the check clone for the five growth characters in the early mature growth phase are given in Table 3.

Tappability

Significantly wide variability was recorded in the wild *Hevea* germplasm, which was reflected in their tappability percentage in the seventh year. This ranged from 0.33 % (AC 3074, AC 3075, AC 3293) to 89.67 % (RO 2727)

Table 2 Analysis of variance for various growth characters in wild *Hevea* germplasm

Source	d.f.	Tappability %—7th year		Girth—7th year		Girth—10th year		Annual girth increment over 3 years		Winter girth increment over 4 years	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F
Genotype	21	2411.82	7.99	260.352	20.128	492.055	21.103	6.931	9.279	0.195	1.964
Replication	2	548.55	1.819	0.404	0.031	1.98	0.085	0.049	0.065	0.015	0.149
Error	42	301.59		12.935		23.317		0.747		0.099	
CD ($P = 0.05$)			28.36		5.93		7.96		1.43		0.52
CD ($P = 0.01$)			38.29		7.93		10.65		1.91		

Table 3 Mean and range of variability for various growth characters in wild *Hevea* germplasm

Character	Wild accessions		General mean	Control		CD (0.05)
	Minimum	Maximum		RRIM 600	Haiken 1	
Tappability percentage—7th year	0.33 (AC 3074, AC 3075, AC 3293)	89.67 (RO 2727)	49.03	64.89	67.96	29.32
Girth (cm)—7th year	22.38 (AC 3293)	53.12 (RO 2727)	43.32	46.83	50.02	5.93
Girth (cm)—10th year	28.33 (AC 3075)	73.51 (RO 2727)	56.71	58.28	67.53	7.96
Annual girth increment (cm/year) over 3 years	1.81 (AC 3075)	6.80 (RO 2727)	4.45	3.81	5.59	1.43
Winter girth increment (cm/year) over 4 years	0.13 (AC 3075)	0.96 (RO 2727)	0.72	1.11	1.10	0.52

Figures in parenthesis denotes the name of accession

while the check clone RRIM 600 and Haiken 1 recorded a tappability of 64.89 and 79.78 %, respectively (Table 3).

Table 4 depicts the absolute mean tappability percentage of accessions over a period of 4 years, starting from seventh year to tenth year after planting and their ranks. High growth-vigour of the accessions in the early growth phase is related to immaturity period. In the present study, out of 18 wild accessions, eight reached tappability in the seventh year, twelve in the eighth year, and fourteen accessions in the 9th year.

Only two more accessions got tappability ranging from 8 % (AC 3074) to 40 % (RO 2948) after the tenth year and two accessions never reached tappability (AC 3075 and AC 3293) at all. The fast-growing accessions—such as RO 2727 (100 %), followed by MT 915 (93 %), MT 2229 (92 %), MT 1020 (81 %), MT 900 (80 %), RO 3197 (80 %), AC 3514 (80 %) and RO 3169 (74 %)—reached the highest tappable percentage as early as in the seventh year. The mean over 4 years and their corresponding ranks revealed that the accession RO 2727 remains in the number one position closely followed by the second rank (MT 900, RO 3197); fourth rank (RO 3169); and fifth rank (MT 915, AC 3514) indicating their overall superiority for growth vigour and tappability.

Girth and growth

Growth behavior of rubber and clonal performance are highly variable under varying agroclimates. In *Hevea*, growth measured in terms of increase in girth of the main trunk remains the most important variable to commercial planters and managers. Girth at age seven is the foremost factor taken into account for evaluating the progress of growth and attainment of maturity for tapping. In the seventh year after planting, the girth of plants ranged from 22.38 cm (AC 3293) to 53.12 cm (RO 2727) with a general mean of 43.32 cm; a similar growth trend was exhibited by these accessions in the tenth year also (Table 3). The growth performance of a clone can also be assessed on the basis of annual girth increment. RO 2727 recorded the highest girth increment (6.80 cm/year) over 3 years, while the check clones RRIM 600 and Haiken 1 recorded an increment of 3.81 and 5.59 cm, respectively.

Winter growth

A low temperature during winter periods hinders growth of *Hevea* plants. Winter girth increment (cm a^{-1}) over 4 years ranged from 0.13 cm/year (AC 3075) to 0.96 cm

Table 4 Mean tappareability percentage and ranks in wild *Hevea* germplasm accessions

Accession/clone	Mean tappareability percentage				Mean over 4 years	Rank
	7th year	8th year	9th year	10th year		
RO 2727	100	100	100	100	100	1
MT 900	80	100	100	100	95	2
RO 3197	80	100	100	100	95	2
RO 3169	74	100	100	100	93	4
MT 915	93	93	93	93	93	5
AC 3514	80	93	100	100	93	5
MT 2229	92	92	92	92	92	8
RO 2908	60	93	100	100	88	10
MT 1020	81	81	89	89	85	13
AC 3810	38	87	100	100	81	14
RO 2638	40	80	100	100	80	15
RO 2886	43	67	93	93	74	16
RO 3043	33	80	87	93	73	17
RO 2901	23	69	100	100	73	18
RO 2948	7	7	23	40	19	19
AC 3074	0	0	0	8	2	20
AC 3075	0	0	0	0	0	21
AC 3293	0	0	0	0	0	21
RRII 203	93	93	93	93	93	5
PB 235	65	87	100	100	88	11
RRIM 600	75	92	92	100	90	9
Haiken 1	80	87	87	87	85	12

a^{-1} (RO 2727). Certain tolerant wild accessions, such as RO 2727, topped the winter girth increment rate (0.96 cm a^{-1}), closely followed by MT 915 (0.95 cm/year), and RO 3197 (0.85 cm/year), all of which have reached early maturity in the seventh year. The check clones RRIM 600 and Haiken 1 recorded a winter girth increment of 1.11 and 1.10 cm a^{-1} , respectively.

Genetic variability

Components of variation and heritability (H^2) in the broad sense were estimated in the population (Table 5). In general, the genetic components for the characters revealed that the magnitude of phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the characters. Wide differences between PCV (50.29) and GCV (24.82) were observed for winter girth increments and tappareability percentage in the seventh year also showed high PCV (64.70) and GCV (54.13), while for the remaining traits the difference between PCV and GCV was low.

Heritability, or the degree of genetic control associated to some interest trait, is one of the most important parameters within the breeding context. The heritability (broad sense) estimates ranged from 24 to 87 %. Girth in

the tenth year after planting showed the highest heritability (87 %) followed by the seventh year (86 %), annual girth increment (73 %), tappareability percentage (70 %), and winter girth increment (24 %).

Correlations

Correlations worked out between the six growth traits revealed that girth was significantly correlated with the other growth traits (Table 6). Annual girth in the seventh year after planting was highly significantly correlated with the annual girth in the eighth year (0.989), annual girth in the ninth year (0.963), annual girth in the tenth year (0.962), annual girth increment (0.65), and winter girth increment (0.612). Similarly, the tenth year annual girth was significantly correlated with the annual girth increment (0.833) and winter girth increment (0.624). Interestingly, annual girth increment was significantly correlated with the winter girth increment (0.489).

Performance

Individual performance of the accessions was assessed by summing up the rank values obtained for each character based on the parametric relation of tree characters to

Table 5 Phenotypic and genotypic coefficients of variation and heritability for quantitative characters

Character	PCV	GCV	Heritability % (H ²)
Tappability percentage—7th year	64.7	54.13	70
Girth—7th year	22.55	20.96	86
Girth—10th year	23.63	22.04	87
Annual girth increment (over 3 years)	37.66	32.26	73
Winter girth increment (over 4 years)	50.29	24.82	24

Table 6 Correlation coefficients among the growth characters in wild *Hevea* germplasm

Character	Annual girth—8th year	Annual girth—9th year	Annual girth—10th year	Annual girth increment over 3 years	Winter girth increment over 4 years
Annual girth—7th year	0.989**	0.963**	0.962**	0.650**	0.612**
Annual girth—8th year		0.990**	0.989**	0.749**	0.593**
Annual girth—9th year			0.999**	0.828**	0.611**
Annual girth—10th year				0.833**	0.624**
Annual girth increment over 3 years					0.489*

*, ** significant at $P = 0.05$ and 0.01 levels, respectively

growth vigour. The accessions were ranked using the three main growth parameters: annual girth (cm) in the tenth year, annual girth increment (cm a^{-1}) over 3 years and mean tappability percentage over 4 years for overall performance. The rank sum values ranged from 3 to 66 with a general mean of 34.23 (Table 7) and top 30 % vigorous accessions could be identified.

Discussion

In the present investigation, the growth pattern/behavior of different wild genotypes under these environmental conditions was examined. The genotypes exhibited highly clonal differences ($P = 0.05$) for all the five quantitative traits studied. Significantly wide variability was observed in this population for various growth characters in the early mature growth phase (Table 3) are discussed under three major grouping characters/heads.

Tappability

By mutual agreement among the rubber-producing countries, which participated in the International Rubber Regulation Agreement (McFadyean 1944), tapping is commenced in an area when 55 % or 130 trees per hectare meet the 45 cm trunk -girth criterion mentioned. Today, a plantation is usually opened for regular tapping when 70 % of the trees attain a trunk girth of 50 cm, at a height of 125 cm (Vijaykumar et al. 2000). However, the practice of tapping trees at a girth of 45 cm is also adopted by some

Table 7 Ranking of wild accessions and clones based on growth parameters

Accession/clone	Rank sum	Rank
RO 2727	66	1
RO 3197	59	2
MT 915	58	3
MT 900	58	3
RO 3169	53	5
MT 2229	45	6
Haiken 1	45	6
RO 3043	39	8
AC 3810	37	9
RRII 203	36	10
RRIM 600	34	11
MT 1020	31	12
RO 2908	31	12
RO 2638	30	14
RO 2886	28	15
AC 3514	24	16
RO 2901	23	17
PB 235	22	18
RO 2948	13	19
AC 3293	11	20
AC 3074	7	21
AC 3075	3	22
General mean 34.23		

large growers in India; it is the standard procedure recommended in Malaysia and by the International Rubber Research and Development Board (IRRDB 1984).

Differences in the growth rate of rubber plants have been observed under unfavorable environmental conditions, such as drought (Chandrasekhar et al. 1996) and cold (Vinod et al. 1996), and it may take seven or more years to reach tappable size (van Schoonneveldt 1949). The adverse environmental conditions prevailing during winter season prolonged the immaturity period in certain *Hevea* clones at Meghalaya region, India (Reju et al. 2001) and China (Huang and Zheng 1983). In traditional rubber-growing areas of India, generally the modern *Hevea* clones achieve tappareability between 7 and 9 years after planting. Under unfavorable climatic conditions, such as low winter temperature in non-traditional areas like Meghalaya, NE India, it takes 9–10 years to attain maturity (Reju et al. 2001) and modern clones in two trials were opened for tapping in the tenth and twelfth year after planting (Reju et al. 2012).

Significantly wide variability was recorded in the wild *Hevea* germplasm, which was reflected in their tappareability percentage in the seventh year. AC 3074, AC 3075, and AC 3293 recorded the lowest tappareability percentage and the RO 2727 (89.67 %) recorded the highest percentage, which is higher than the standard check clone RRIM 600 and Haiken 1 (Table 3). In rubber, a clonal difference for this trait was also observed by other scientists (Vinod et al. 1996; Reju et al. 2001).

High growth-vigour of the accessions in the early growth phase is related to immaturity period. In the present study, out of 18 wild accessions, eight reached tappareability in the seventh year, twelve in the eighth year and fourteen accessions in the ninth year (Table 3). In addition to the above, only two more accessions got tappareability ranging from 8 % (AC 3074) to 40 % (RO 2948) after the tenth year and two accessions never reached tappareability (AC 3075 and AC 3293) at all, indicating their susceptibility to cold stress. Vinod et al. (1996) reported that in a performance study of 15 oriental clones in Tripura, NE India, even after the twelfth year after planting all the trees in the two clones (Gl 1 and Harbel 1) had not reached tappareability.

The fast growing accessions—such as RO 2727 (which recorded 100 % tappareability) followed by MT 915 (93 %), MT 2229 (92 %), MT 1020 (81 %), MT 900 (80 %), RO 3197 (80 %), AC 3514 (80 %) and RO 3169 (74 %)—reached the highest tappable percentage as early as in the seventh year. The mean over 4 years and their corresponding ranks revealed that the accession RO 2727 remains in the number one position, closely followed by the second rank (MT 900, RO 3197), fourth (RO 3169), and fifth rank (MT 915, AC 3514) indicating their overall superiority for growth-vigour and tappareability. Even in the traditional rubber-growing areas of India, a performance study of five introduced elite IRCA clones and a popular control clone RRIM 105 revealed that out of the five, two clones attained tappable girth in the eighth year, three

clones in the ninth year and the control clone in the tenth year (Reghu et al. 2008). Moreover, delay in tappareability was more pronounced in the non-traditional, drought-prone North Konkan sub-humid region, where under rain-fed conditions the immaturity period is more than 9 years (Chandrasekhar et al. 1998), 9–10 years at Meghalaya (Reju et al. 2001).

More emphasis is needed for identification of vigour genotypes in order to breed clones for early tappareability. Incorporation of these wild selections in breeding for growth vigour in cultivated clones will certainly pave the way for attainment tappable girth one to 2 years earlier, thereby increasing plantation profitability. Barlow and Ng (1966) have shown that based on projections of rubber prices fluctuating from \$1.2 to \$1.60 per kilo, gains of up to \$1160 per ha could be obtained by reducing the period of immaturity by even 6 months.

Girth and growth

Influence of climate on the growth of rubber trees is cumulative due to the long duration of the crop. In *Hevea*, breeding is aimed at synthesis of ideal clones with high production potential to combine with desirable secondary attributes like initial vigour, high growth rate, and smooth thick bark. Moreover, the identification and availability of specific genotypes for use in breeding programmes assumes much significance, since a wide array of diverse wild accessions and species of *Hevea* are available only in the Amazon forests of Brazil, the centre of diversity of the genus.

Biological growth, which is regulated by genetics and environment, changes continuously with age, leading to an increase in volume, size, or shape of organism. In *Hevea*, girth at age seven is the foremost factor taken into account for evaluating the progress of growth and attainment of maturity for tapping. Girth and rate of girth increase are also used in experimental work to assess the growth performance of new planting materials and effects of cultural treatments on growth (Shorrocks et al. 1965; Chandrasekhar et al. 1998). The wild accession RO 2727 recorded the highest girth from the seventh year to the tenth year (Table 3).

The growth performance of a clone can also be assessed on the basis of annual girth increment (GI), which is seen around the year in rubber. RO 2727 recorded the highest girth increment (6.80 cm a^{-1}) over 3 years, compared to the check clones RRIM 600 and Haiken 1. Several researchers have also reported variations in the wild germplasm with respect to certain early growth traits and growth-rate in the traditional rubber-growing regions in India (Rao et al. 2011; Rao and Varghese 2011; Rao and Reghu 2012; Rao et al. 2013a) as well as in the non-traditional (Rao et al. 2006; Krishan et al. 2011; Rao et al. 2013b).

In general, during the non-winter season (Weather Regime I), all the wild accessions and clones under study attained the maximum level of growth, as compared to the winter season (Weather Regime II). Since it is the first report on the performance of wild accessions in India, no such information is available so far. But on various modern *Hevea* clones, certain similar observations were reported earlier in the northeast India (Meenattor et al. 1991; Reju et al. 2000, 2001). Growth evaluation of leaven clones by Gohain et al. (2004) in the Dooars region of West Bengal revealed that the growth of the clones varied significantly from the second year to the eighth year, with the highest growth recorded by RRII 208 (46.53 cm) and RRIM 600 (46.50 cm). High growth indices were recorded by the clones RRIM 600, RRII 208, RRII 105 and PB 235 and identified as better adaptable clones. Better performance of PB 235, RRIM 600, RRII 105 and RRII 203 was also reported from Agartala, Tripura, NE India (Vinod et al. 1996).

Characteristics, such as girth and GI before and after tapping, determine the age of attainment of tappability and the timber value. Apart from shortening the gestation period, if the initial vigorous growth is sustained in the mature phase, then it also significant in the context of the increasing importance of rubber wood timber (Rao et al. 2013b). These high-growth selections are very useful in improving the cultivated tree girth in India. Interestingly, a study made in China led to selection for girth at the age of 5 years from the IRRDB *Hevea* germplasm, which resulted in outstanding, vigorous clones (Husan et al. 2002). Clearly, trees that reach tappable girth earlier, that is, vigorous trees, tend to yield better than those that reach tappable girth later (Nugawela et al. 2002).

Winter growth

The *Hevea* plant requires a mean monthly air temperature ranging between 25 and 28 °C for its optimum growth (Rao and Vijayakumar 1992). In the study area, from November to March the winter temperature range is 12–15 °C. The winter growth is indicative of a genotype's ability to continue its metabolic functions under stress and hence could be an indication of its tolerance to cold stress in the current study. Certain tolerant wild accessions, such as RO 2727, MT 915, and RO 3197, with high winter growth rate over 4 years are on par with the check clones (RRIM 600 and Haiken 1), which also reached early maturity in the seventh year. Growth of accessions during winter period was low as compared to the non-winter period and this is in line with an earlier report. Cold stress has been reported to inhibit growth of *Hevea* plants (Jacob et al. 1999; Alam et al. 2005). In

Agartala, NE India, the overall growth inhibition was 20 %, when compared to the traditional region (Sethuraj et al. 1991), which is a major concern for researchers studying the NE regions of India.

Almost three wild accessions—including AC 3075, AC 3293, and AC 3074— showed negligible growth, an indication of high susceptibility to winter stress. Meanwhile, the threshold temperature for growth is believed to be in the range of 20 °C (Jiang 1988). Photosynthesis is impaired at 10 °C (Huang and Zheng 1983). During winter in the northern part of West Bengal, plants are exposed to temperatures as low as 5 °C followed by moderately high temperature during the day, sometimes causing irreversible frost-damage (Xu and Pan 1990).

Photo-inhibition of photosynthesis, induced by high photon flux, has been reported in the plants experiencing abiotic stress like low temperature (Sathick et al. 1998). There was about a 50 % reduction in net photosynthetic rate in winter compared to the pre-winter and post-winter periods in young *Hevea* plants in field condition (Dey and Jacob 2010).

Because of the cold stress and photo-inhibition of photosynthesis, plants might have utilized their reserved energy resulting in no growth or low growth in the plants. Hence, winter growth is the critical criteria for the real assessment of a clone in terms of cold tolerance. During this period, a wide range of differences were observed in rubber: the plant growth was completely stopped at North Konkan region (Chandrashekar et al. 1996), no growth occurred in most of the clones in northern region of West Bengal (Meti et al. 1999), and only 4 % growth was observed in *Hevea* at Tura, Meghalaya (Thapliyal et al. 2011).

Three wild accessions (AC 3075, AC 3293, AC 3074) are highly susceptible to winter stress, and as a result of this, they never reached tappable girth even after the ninth year after planting. Even though in the tenth year after planting, the susceptible accession AC 3074 recorded only 8 % of tappable trees, and the other two accessions (AC 3293, AC 3075) never attained maturity. Vinod et al. (1996) also reported that in a performance study of 15 oriental clones in Tripura, NE India, even after the twelfth year, all the trees in the two clones (GI 1 and Harbel 1) had not reached tappability. In the present investigation however, certain accessions (RO 2727, MT 915, and RO 3197) performed better during the winter and showed a higher overall growth and tappability, indicating the adaptability of these accessions to cold stress. Similar observations on modern clones were also reported from the sub-Himalayan West Bengal region of India (Gohain et al. 2004) and China (Huang and Pan 1979; Jiang 1988).

Genetic variability

Genetic components for the characters revealed that the magnitude of phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the characters (Table 4), denoting the environmental factors that influence in expression of these traits. Wide differences between PCV (50.29) and GCV (24.82) were observed for winter girth increment, which suggested a greater role for the environment in the expression of the trait. Tappability percentage in the seventh year also showed high PCV (64.70) and GCV (54.13), while for the remaining traits, the difference between PCV and GCV was low indicating the lesser role of environment in the expression of these traits. Higher GCV and PCV indicated sufficient variability in this population for crop improvement for these traits. Rao et al. (2011) and Rao et al. (2006) also reported similar results while studying the wild *Hevea* germplasm in traditional and drought-prone regions in India.

Heritability indicates how much of the phenotypic variability has a genetic origin, and gives objective information for the genetic selection process (Falconer 1981). The amount of advance to be expected from selection can be achieved by estimating heritability along with coefficient of variability. Burton (1952) also suggested that GCV and heritability estimates would give better information of the extent of advance to be expected by selection. The highest broad-sense heritability exhibited for girth in the tenth year followed by the seventh year, annual girth increment, and tappability percentage. Except for winter girth increment, all other traits showed high H^2 , indicating that high proportion of variation is due to genetic inheritance. Rao et al. (2006) and Rao et al. (2011) have reported moderate to high H^2 in the wild *Hevea* germplasm with respect to certain growth parameters, like girth and girth increment, in traditional and non-traditional areas in India.

Correlations

Correlation estimates between girth at different ages (i.e. from the seventh to the tenth year) and other growth traits are useful in selection of desirable plant types in designing an effective breeding programme. Growth is a complex phenomenon encompassing the interactions between many morphological traits. Therefore, selection should be based on these traits and their correlation with girth.

Correlations worked out between the six quantitative traits revealed that girth was significantly correlated with the other growth traits (Table 6). One of the important features is that the correlation strength between the growth traits increases with increasing age: between years seven to eight (0.989), between years eight to nine (0.990), between years nine to ten (0.999). A similar increase in correlation

strength from the seventh to the tenth year was also noticed for annual girth increment (0.65–0.833) and winter girth increment (0.612–0.624).

In rubber plants, significantly increasing trend of correlations combined with increase in plant age, and girth growth character are observed. Girth was one of the main traits for selection of a desirable genotype. Rao and Varghese (2011), Rao et al. (2011), and Rao and Reghu (2012) also reported positive correlations between girth, girth increment, and plant height in wild *Hevea* germplasm in the traditional rubber growing area of Kerala, India. Rao et al. (2006) and Krishan et al. (2011) reported significantly positive correlation between girth and girth increment while studying different sets of wild *Hevea* germplasm in a drought-prone region of India.

Performance

Based on the three main growth parameters—annual girth (tenth year), annual girth increment, and tappability percentage—individual performance of each accession was assessed in terms of overall performance (Table 7). The top 10 vigorous accessions—such as RO 2727, RO 3197, MT 915, MT 900, RO 3169, MT 2229, RO 3043, AC 3810, and MT 1020—could be identified as potentially superior for various growth traits, also considered as best ecotypes. Balasineha et al. (1988) in Cocoa and Mercy (2001); Rao et al. (2006), (2011); Rao and Reghu (2012); and Rao et al. (2013b) also reported similar rankings in wild *Hevea* accessions, respectively while evaluating the Brazilian germplasm in India.

Conclusion

Rubber being a perennial crop, repeated measurements from the same tree over several years are possible. In the case of girth, repeated measurements could be analysed to study the growth pattern of different clones and be used to group the clones (Winitha Margret et al. 1988). It will be useful to the *Hevea* breeder in assessing the genotypes for vigour and arranging them into groups with similar growth patterns for selection.

The present study confirmed the presence of wide variability in the germplasm for various growth traits. A group of accessions—RO 2727, RO 3197, MT 915, MT 900, RO 3169, MT 2229, RO 3043, AC 3810 and MT 1020—could also be formed as best ecotypes, ranked top in the cold-prone sub-Himalayan region. Attainment of the highest mean girth in the early period, which led to early tappable stage of these accessions, could be explained by its high growth rate when compared with the other wild accessions. It also possesses high timber potential. The

superior growth performance indicated that these accessions may be physiologically better adapted to the subtropical climate. The advantages of these ecotypes may be explained by their inherent genetic potential and the genetic variability among accessions may be due to factors like heterogeneity, selection pressure under diverse environments, genetic drift and geographical origin.

These accessions could be used to increase the growth rate in elite clones, which lead to the early maturity of a crop. Since they are genetically diverse from the cultivated clones, transgressive segregation and heterosis can be expected on crossing these accessions with elite Wickham cultivars. These selections have potential for the development of cold-tolerant clones for these regions and also in broadening the genetic base of present-day cultivated rubber. The early growth of planting materials derived from these selections would help in tiding over chilling or cold damage as well during low winter temperature periods.

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