Variability in Wild Germplasm of Natural Rubber (*Hevea brasiliensis* Muell. Arg.)

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Abstract

Para rubber (*Hevea brasiliensis*), a native of the Amazon forests of South America, is the chief source of natural rubber in the world. With the objective of broadening the original gene pool collected by Sir Henry Wickham in 1876, the International Rubber Research and Development Board (IRRDB) made a large collection of wild germplasm from Acre, Rondonia and Mato Grosso states of Brazil in 1981, which was distributed to various member countries including India for conservation and evaluation. In the present study, variability was assessed in a set of 195 *Hevea* accessions belonging to the wild gene pool in India, using 22 characterization descriptors and eight quantitative growth characters in the juvenile stage. The Shannon-Weaver diversity indices worked out for each of the 22 qualitative traits indicated a high level of diversity in the collection. The range of variation for quantitative traits *viz*., plant height (0.43 to 3.16 m), basal diameter (0.81 to 2.52 cm), number of whorls (1.23 to 5.48), inter-node length (4.11 to 45.26 cm), number of leaves (11.44 to 91.26), single leaf area (80.22 to 223.57 cm^2), total leaf area (2356.00 to 14660.50 cm^2) and leaf area index (0.24 to 1.47), also represented wide variability in the collection. The accessions were ranked for overall performance and top 10% were selected for early growth vigour. The high level of variability observed in this germplasm indicates its potential use in crop improvement programmes and for broadening the genetic base of *Hevea*.

Key words: Hevea brasiliensis, Shannon-Weaver diversity index, wild rubber germplasm.

Introduction

The para rubber (*Hevea brasiliensis* Willd. ex Adr. de Juss.) Muell. Arg. tree is a native of the hot damp forests of the Amazon, South America. One of the most important commercial crops of Asia and Africa, it is the chief source of natural rubber (NR), recovered from the latex of the plant, and finds use in about 50,000 products the world over. The crop is cultivated in over 11.40 million ha in the world with a production of about 11 million tonnes in 2012.

Rubber was introduced to tropical Asia by Sir Henry Wickham in 1876 through seeds brought to Kew Botanical Gardens in the UK. The entire development of rubber plantations in Asia is attributed to this collection made from Boim near the Tapajos river in Brazil (Lane, 1953; Wycherly, 1968). However, this area represents only a minuscule of the entire geographical range of *Hevea brasiliensis* in the Amazon forests of Brazil (Wycherly, 1968; Schultes, 1977; Allen, 1984). Using this small gene pool, a substantial ten-fold improvement in productivity was achieved within a century. However, the genetic advance gained in the early breeding phases seems to have slowed down in the more recent phases of breeding (Tan, 1987; Ong and Tan, 1987; Simmonds, 1989) for which the narrow genetic base is considered to be one of the major reasons. The original narrow base was further reduced through the unidirectional selection for yield, a cyclical generation wise assortative mating pattern (GAM) (where the best clones in each cycle are used as parents for the next), and wide adoption of clonal propagation. Further collection and conservation of the wide variability in the Brazilian rain forests was carried out in 1945 and a few sporadic collections were made over the next few years. Considering the urgent need for conserving the dwindling genetic resources in the Amazon forests, and for broadening the genetic base, a joint expedition of the International Rubber Research and Development Board (IRRDB) and the Brazilian Government was organized in 1981 to collect fresh wild germplasm from the center of diversity in Brazil. The team collected 64,736 seeds and budwood from 194 presumably high yielding mother trees from the states of Acre, Mato Grosso and Rondonia (Ong *et al.,* 1983; Mohd Noor and Ibrahim, 1986). India received a share of these collections and a total of 4548 accessions have been established in traditional and non-traditional areas in conservation-cum-source bush nurseries.

Materials and Methods

A set of 195 accessions and four control clones (RRIM 600, PB 235, RRII 208, RRII 105) of *Hevea brasiliensis* planted in 2003 at the Central Experiment Station of the Rubber Research Institute of India, Chethackal, Pathanmthitta District, Kerala, were used for this study. These accessions were planted in an augmented randomized block design in five blocks, with a plot size of five and a spacing of 1 x 1 m. Provenance-wise distribution of accessions is presented in *Table 1*. The wild accessions were characterized at the age of one year using a set of 22 qualitative descriptors designed for rubber (Rao *et al.,* 2005) *viz*., plant height, stem girth, axillary bud, leaf scar, leaf storey/whorl shape, separation of storey, external appearance of whorl, leaf pulvi-

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nous, petiole shape, petiole size, petiole orientation, petiolule orientation, leaf lamina colour, leaf lamina lustre, leaf texture, leaf blade shape, leaf blade margin, leaf blade size, leaf cross sectional appearance, leaf apex, leaf blade surface and colour of veins. Genetic diversity for each of the 22 descriptors was estimated using the Shannon-Weaver Diversity Index (SDI). The SDI was computed using the formula

$$H' = -\sum_{i=1}^{s} p_i \log_e (p_i)$$

where s is the number of phenotypic classes or descriptor states for a given qualitative descriptor and p is the proportion of the total number of accessions in the i-th class (JAIN *et al.,* 1975). The index was standardized to keep its value in the range 0 to 1 by dividing the value by $\log_e s$ (POOL, 1974). The estimate of diversity was from 0 to 1.0, with 0 indicating no variation and 1, as the maximum variation.

In addition to the qualitative traits mentioned above, eight quantitative characters were evaluated as follows:

(1) Plant height from the bud union to the tip of the plant (m).

(2) Diameter of the plant at 30 cm height from the bud union (cm) by vernier calipers.

(3) Number of leaf whorls

(4) Internode length (distance between the base of the top most mature whorl and the first leaf in the whorl) (cm).

(5) Number of leaves

(6) Single leaf area (cm^2)

(7) Total leaf area (cm^2)

(8) Leaf area index

Analysis of variance (ANOVA) was carried out for all the eight quantitative characters, as prescribed by SHARMA (1998). The overall performance of all these accessions were assessed by rank sum method (KANG, 1988) using these eight traits. Based on the mean value of a character, each accession was ranked, giving rank "1" to the best performer. Then the ranks across all traits for each accession were pooled to give a rank sum. Hence the lowest rank sum indicated the best performer.

Results and Discussion

Diversity in the Hevea germplasm

The Shannon – Weaver information index provides a measure of phenotypic diversity based on frequency data, and therefore takes into account number of alleles in the population (allele richness) and gene frequencies (allelic evenness). Phenotypic diversity in terms of the Shannon –Weaver diversity index for each of the 22 qualitative traits showed different levels of diversity for the traits. The characters for which high diversity was observed include plant height, stem diameter, leaf storey shape, separation of storey, petiole shape and size, petiolule orientation, leaf lamina lustre, margin, leaf apex, cross sectional appearance and leaf blade surface at 0.24–0.99 SDI (*Table 2*). This information can be used in identification of potential wild accessions for crop improvement programmes.

Variability for this collection was also reflected in the eight additional traits which are a measure of the vigour of an accession in the early growth phase and hence related to immaturity period. Apart from shortening the gestation period, if the initial vigorous growth is sustained in the mature phase, then it assumes significance

Table 1. – Provenance wise distribution of wild *Hevea* accessions.

Provenance	District	No. of accessions
Acre	1) Brasileia (B)	24
	2) Feijo (F)	13
	3) S. Madureira (S)	26
	4) Tarauaca (T)	12
	5) Xapuri (X)	3
	Others	1
	Total	79
Rondonia	1) Ariquemes (A)	7
	2) Calama (C)	6
	3) Costa Marques (CM)	29
	4) Jaru (J)	15
	5) Jiparana (JP)	7
	6) Ouro Preto (OP)	1
	7) Pimenta Bueno (PB)	17
	Total	82
Mato Grosso	1) Aracatuba (C)	10
	2) Itauba (IT)	21
	3) Vila Bela (VB)	2
	Total	33
Others		1
Grand total		195

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Table 2. – Shannon-Weaver Diversity Index (SDI) for 22 characters.

Descriptor	SDI	Descriptor	SDI
Margin	0.9999	Angle orientation	0.4447
Leaf blade surface	0.8751	Leaves size	0.4441
Height	0.8591	Leaves shape	0.4209
Petiole size	0.8038	External appearance	0.4196
Leaf blade luster	0.7586	Axillary bud	0.1944
Separation of whorls	0.7485	Colour of veins	0.1944
Petiole shape	0.7319	Leaf blade colour	0.1416
Leaf storey shape	0.6891	Leaf blade texture	0.04541
Petiolule orientation	0.6851	Leaf scar	0.04541
Girth	0.5815	Leaf apex	0.511
Cross sectional appearance	0.5413	Leaves-pulvinous	0.235

in the context of the increasing importance of rubber wood timber. ANOVA revealed significant differences among the accessions for traits plant height, stem diameter, number of whorls, inter-node length, single leaf area, total leaf area and leaf area index.

The range and general mean of eight characters in comparison with the controls is given in *Table 3*. Plant height ranged from 0.43 m–3.16 m with a general mean of 2.05 m. Four significant accessions to control clone RRII 105 (1.99 cm) were RO 2793 (3.16 m), AC 1822 (3.02 m), RO 4428 (3.02 m) and AC 4300 (2.93 m). Rao *et al.* (1999) and Abraham (2001) had also reported similar results in two sets of wild genotypes at the juvenile phase, where the mean heights of some wild genotypes were higher than the popular check clone RRII 105. This indicates presence of variability for this trait which can be used to improve the vigour of cultivated clones. Medium to high plant height combined with high branching is desirable for selection of potential timber/timber latex clones.

Stem diameter, which is another indicator of the growth rate, also exhibited a wide variability in the wild population and ranged from 0.81–2.52 cm with the general mean of 1.68 cm. Ten accessions recorded significantly higher stem diameter than the popular control RRIM 600 (1.63 cm), AC 2930 (2.52 cm), RO 4428 (2.5 cm), AC 1849 (2.46 cm), AC 160 (2.42 cm), AC 4280 (2.40 cm), AC 4300 (2.39 cm), MT 4243 (2.38 cm), RO 3429 (2.36 cm), RO 2793 (2.32 cm) and AC 4394 (2.30 cm). These accessions are very useful in improving the cultivated tree girth. In China, selection for girth at the age of five years from the IRRDB *Hevea* germplasm resulted in outstanding vigorous clones (Husan *et al.*, 2002).

RO 2793 recorded the maximum number of whorls per plant (5.48) and AC 2596 the minimum (1.23) with a general mean of 3.32. RO 2793 (5.48), recorded significantly higher number of whorls than the best control clone RRIM 600 (3.84). Rate of production of leaves is also an indicator of the vigour of the plants in the active growth phase. Along with plant height and stem girth, foliar characters like number of leaves and leaf flushes produced give a fair indication of the vigour of the clones in its juvenile phase. Knowledge of such traits helps the breeder to apply appropriate selection index for yield contributing morphological characters.

Internode length of the plants ranged from 4.11–45.26 cm with a general mean of 25.08 cm. Long internodes were recorded in AC 154 (45.26 cm), RO 10 (42.59 cm),

Table 3. – Range and general mean of quantitative growth characters.

Character	Minimum		Maximum		General mean	Controls				CD
	Accession	Value	Accession	Value		RRII 105	RRIM 600	PB 235	RRII 208	
Plant height (m)	MT 50	0.43	RO 2793	3.16	2.05	1.99	2.47	2.66	2.07	0.925
Stem diameter (cm)	AC 3642	0.81	AC 2930	2.52	1.68	1.58	1.63	2.044	1.45	0.658
No. of whorls	AC 2596	1.23	RO 2793	5.48	3.32	3.40	3.84	3.64	3.75	1.352
Inter node length (cm)	AC 2596	4.11	AC 154	45.26	25.08	17.88	16.88	19.00	16.32	11.107
No. of leaves	AC 2596	11.44	AC 159	91.26	49.57	51.24	65.28	65.44	61.15	NS
Single leaf area (cm^2)	RO 4314	80.22	AC 2930	223.57	109.86	94.12	69.42	93.58	89.37	32.66
Total leaf area (cm^2)	AC 2596	2356.00	RO 3803	14660.50	5486.47	4842.45	4464.56	6024.68	5568.99	4608.06
Leaf area index	AC 2596	0.24	RO 3803	1.47	0.55	0.48	0.45	0.60	0.56	0.460

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AC 3947 (41.89 cm), RO 2252 (40.89 cm), RO 8 (40.59 cm), AC 4300 (40.54 cm) compared to the best control PB 235 (19 cm). Twenty six accessions recorded significantly higher values than PB 235. Well-separated leaf storeys are desirable for efficient trapping of sunlight, thereby leading to higher growth.

The total number of leaves produced also showed a wide range of variation from 11.44 to 91.26 with a general mean of 49.57. The maximum number of leaves was recorded in AC 159 (91.26) followed by AC 4149 (78.46), MT 4371 (77.81), MT 64 (77.46), AC 176 (75.46) and RO 3246 (73.76) as compared to the control clone PB 235 (65.44).

A wide range (80.22–223.57 cm^2) was observed for leaf size in terms of single leaf area, with general mean of 109.86 cm^2. Top five accessions with large leaves were AC 2930 (223.57 cm^2), AC 2203 (219.27 cm^2), RO 3803 (217.72 cm^2), 7-102 (203.97 cm^2) and RO 3857 (203.345 cm^2). The control clones in general, had smaller leaves than the wild accessions, ranging from 69.42 cm^2 (RRIM 600) to 94.12 cm^2 (RRII 105). One hundred and forty five accessions recorded significantly higher leaf size than RRII 105.

Total leaf area (TLA) of the plant was estimated from the total number of leaves and single leaflet area. The mean values of the accessions ranged from 2356.0–14660.5 cm^2 with a general mean of 5486.47 cm^2. The maximum leaf area was recorded in RO 3803 (14660.5 cm^2), AC 2930 (13632 cm^2), AC 4095 (13081 cm^2). Among the controls PB 235 (6024.68 cm^2) recorded the maximum. 20 accessions had significantly higher total leaf area than the best control clone PB 235. DEY *et al.* (1995) reported the positive relation of TLA with the girth of the plant.

Leaf area index (LAI) ranged from 0.24–1.47 with general mean of 0.55. Maximum index was noted in RO 3803 (1.47) followed by AC 2930 (1.36), AC 1822 (1.32), AC 4095 (1.31), AC 176 (1.25), 7-102 (1.24) and RO 87 (1.24). Among the controls, the index ranged from 0.45 (RRIM 600) to 0.60 (PB 235) and 20 accessions recorded significantly higher index compared to the best control. ISHII (1998) had considered leaf area index as one of the major contributing parameters of canopy photosynthesis. High growth rate is usually associated with high leaf area index in crop plants, and hence high LAI indicates a high rate of photosynthesis (WANG *et al.,* 1991). However, there is an optimum index where crop growth rate reaches maximum level. Leaf area index and leaf orientation have been identified as two important factors which determine growth in pre-exploitation stage (SETHURAJ, 1985; DEY *et al.,* 1995).

Provenance-wise performance is shown in *Table 4*. Among the three provenances, the mean plant height was the maximum for Rondonia (2.00 m) followed by Mato Grosso (1.99 m) and Acre (1.91 m), while the controls recorded a mean of 2.66, 2.47, 2.07, and 1.99 m for PB 235, RRIM 600, RRII 208, and RRII 105 respectively. The mean stem diameter was the maximum for Acre (1.73 cm) followed by Rondonia (1.71 cm) and Mato Grosso (1.66 cm) while the controls recorded a mean of 2.04 cm (PB235), 1.63 cm (RRIM 600), 1.58 cm (RRII 105) and 1.45 cm (RRII 208). A study on morphological characterization of the wild germplasm in the juvenile phase in a set of wild accessions in Ivory Coast by CHAPUSET *et al.* (1995) recorded a better yield and timber production potential of accessions from Mato Grosso than those from Rondonia and Acre. Among the three provenances observed, the mean number of whorls was the maximum for Rondonia (2.00 m) followed by Mato Grosso (1.99 m) and Acre (1.91 m) while the best control recorded a mean of 2.66 (PB 235). Mean internode length was maximum for Rondonia (22.99 cm) followed by Mato Grosso (23.50 cm) and Acre (22.51 cm) while the best control recorded a mean of 19 cm (PB 235).

Mato Grosso (51.94) recorded the maximum number of leaves followed by Rondonia (49.70) and Acre (43.44) while the controls recorded a mean of 65.44 (PB 235) and 65.28 (RRIM 600) (*Table 4*). Mean of single leaf area per plant was the maximum for Acre (144.31 cm^2) followed by Rondonia (143.93 cm^2) and Mato Grosso (129.80 cm^2) while the controls recorded a mean of 94.12 cm^2 (RRII 105) and 93.58 cm^2 (PB235). Among the three provenances the mean total leaf area per plant was the maximum for Rondonia (8253.82) followed by Mato Grosso (7723.73) and Acre (7551.16) while the best control recorded a mean of 6024.68 cm^2 (PB 235). Leaf area index was the maximum for Rondonia (0.825) followed by Mato Grosso (0.772) and Acre (0.755) while the control recorded a mean of 0.60 in PB 235 (*Table 4*).

Performance

Individual performance of the accessions was assessed by summing up the rank values obtained for each char-

Table 4. – Provenance wise performance of variability.

Provenance	Plant height (m)	Stem diameter (cm)	No. of whorls	Inter node length (cm)	No. of leaves	Single leaf area (cm^2)	Total leaf area (cm^2)	Leaf area index
Acre	1.91	1.73	3.09	22.51	43.44	144.31	7551.16	0.755
Mato Grosso	1.99	1.66	3.47	23.50	51.94	129.80	7723.73	0.772
Rondonia	2	1.71	3.41	22.99	49.70	143.93	8253.82	0.825
Controls								
RRII 105	1.99	1.58	3.4	17.88	51.24	91.12	4842.40	0.480
RRIM 600	2.47	1.63	3.84	16.88	64.28	69.41	4464.50	0.450
PB 235	2.66	2.04	3.64	19.00	65.44	93.58	6024.70	0.600
RRII 208	2.07	1.45	3.75	16.32	61.15	89.37	5568.90	0.560

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Table 5. – Top ranking wild accessions based in relation to growth.

Accession	Rank sum	Rank
AC 1822	80	1
AC 2930	82	2
MT 4243	114	3
RO 142	122	4
RO 87	136	5
RO 3246	139	6
RO 2750	139	6
RO 3803	144	7
AC 176	160	8
RO 3859	160	8
AC 4095	169	9
RO 3429	183	10
AC 2203	186	11
RO 3262	197	12
MT 4225	200	13
RO 3799	206	14
MT 4254	211	15
MT 200	218	16
RO 3661	221	17
RO 3157	221	17
AC 4394	241	18
AC 4238	244	19
RO 4003	252	20
Bottom		
RO 2286	1100	173
AC 3836	1120	174
AC 4258	1136	175
AC 2596	1150	176
AC 4510	1168	177
General mean = 596.08		

acter based on the parametric relation of tree characters to growth vigour. The rank sum ranged from 80–1168 with the general mean of 596.08. The highest rank sum was in genotype AC 4510 (1168) and AC 1822 showed the lowest rank sum (80) being the best one (*Table 5*). Out of 195 accessions studied, 50.76 per cent recorded a rank sum above general mean showing relatively vigorous growth with respect to growth parameters. Among the three provinces, 60.75 per cent accessions in AC, 48.48 per cent accessions in MT and 42.69 per cent accessions in RO were above the rank-sum general mean. MERCY (2001) and RAO and VARGHESE (2011) have also reported similar studies for ranking in wild *Hevea* accessions respectively. Top 10 per cent rankers identified as best performers are AC 1822, AC 2930, MT 4243, RO 142, RO 87, RO 2750, RO 3246, RO 3803, RO 3859, AC 176, AC 4095, RO 3429, AC 2203, RO 3262, MT 4225, RO 3799, MT 4254, MT 200, RO 3157 and RO 3661. These accessions could be used to increase the growth rate in elite clones. 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 accessions will also serve to broaden the genetic base of cultivated *Hevea* clones.

Conclusion

Genetic variability among accessions may be due to factors like heterogenity, selection pressure under diverse environments, genetic drift and/or geographical origin. A vigorous habit in the early growth phase of the tree reduces the immaturity period. In general, yield and vigour in *Hevea brasiliensis* are hardly separable (SIMMONDS, 1989). Various morphological, anatomical, biochemical and physiological characters of the rubber tree are ultimately manifested in the volume of latex obtained by tapping and the quantum of rubber it contains (POLLINERE, 1966). Vigorous accessions with high variability identified are thus useful for improving early growth vigour with the objective of reducing the immaturity period.

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