

Potential Genotypes of the 1995 RRIM Hevea Germplasm Collection for Future Rubber Breeding and Selection Programme

A.F. ADIFAIZ^{1#}, N.A. MAIDEN¹, N. AIZAT SHAMIN¹, A.G. ZARAWI¹ AND M.Y. RAFII²

Malaysia received a large share of the Hevea germplasm collection from the 1995 Rubber Research Institute of Malaysia (RRIM) expedition. About 50,000 wild genotypes of eight Hevea species originating from the state of Amazonas of Brazil are conserved ex-situ in Rantau Panjang Reserve Forest, Batu Arang, Selangor. As management of such massive numbers of individuals covering extensive areas is demanding, the wild genotypes planted in this germplasm are evaluated in a phased manner. A total of 5,789 wild genotypes of six Hevea species (Hevea brasiliensis, Hevea spruceana, Hevea guainensis, Hevea nitida, Hevea benthamiana and Hevea pauciflora) originating from different locations in the Amazonas, planted in Phase 1 area (58.88 ha) of this germplasm were assessed for their main characteristics specifically latex yield, clear bole volume, girth at opening, annual girth increment and virgin bark thickness.

In general, the wild genotypes of the 1995 RRIM Hevea germplasm produced a considerably low amount of latex yield. Up to 80.84% of these materials were found to produce latex yield of less than 25.00 g/t. Nevertheless, Hevea brasiliensis demonstrated superiority compared with the other five Hevea species in this germplasm. Selection of potential genotypes in this germplasm was to gather good performance genotypes as well as to retain as much genetic variability possible. This process was performed using multiple characteristics selection index on latex yield, clear bole volume and girth at opening. The selection process resulted in a working collection consisting 616 potential genotypes of mainly Hevea brasiliensis. Assessment of major leaf diseases revealed that most of the selected genotypes were free from or only lightly affected by Oidium and Colletotrichum secondary leaf fall while all of them were completely free from Corynespora leaf disease and Fusicoccum leaf blight. These selected materials will be used for further evaluation, genetic improvement and incorporation in the Malaysian Rubber Board's rubber breeding programme for the development of superior latex timber clones.

Keywords: 1995 RRIM Hevea germplasm; genetic resources; Hevea spp.; rubber breeding; selection index

Rubber tree (*H. brasiliensis*), which originates from the Amazon Basin, South America, was first introduced to the East by Sir Henry Wickham in 1876. About 70,000 seeds were collected from Boim near Tapajos River in Brazil and shipped to the Kew

Botanical Gardens near London, of which about 2,700 seeds were germinated. Almost 2,000 seedlings were then dispatched to Ceylon in 1877. Among these, 22 seedlings were shipped to Singapore; half were planted there and nine in Kuala Kangsar, Malaya¹.

¹Production Development Division, Malaysian Rubber Board (MRB), 47000 Sg. Buloh, Selangor, Malaysia.

²Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia.

[#]Corresponding author (e-mail: adifaiz@lgn.gov.my)

Progress of the natural rubber industry over the last 130 years in Asia and Africa has been tremendous. In Malaysia, approximately 1.07 million hectares have been planted with rubber despite the fact that it was founded on a narrow genetic base².

Broadening this genetic base is one of the key areas in rubber breeding. In Malaysia, a number of introductions *viz.* 1951-1952 and 1966, were made to widen the genetic base and sustain further genetic improvements in rubber. Unfortunately, newly introduced South American clones were of a relatively small sample size with low yield, hence, were less frequently exploited in cross breeding³. Some of these introduced genetic materials namely FX 25, FX 3899, IAN 717 and IAN 873 were incorporated into the Rubber Research Institute of Malaysia (RRIM) breeding programme. Several of the crosses were promising and have contributed in the development of RRIM 900 and RRIM 2000 series clones⁴. In addition, exchanges of improved genetic materials among rubber growing countries were also carried out notably in 1954 and 1974⁵.

Further work on broadening the genetic base was strengthened with the germplasm collection in 1981 by the International Rubber Research Development Board (IRRDB) and the Brazilian government in three western states of Brazil, namely, Acre, Rondonia and Matto Grosso. This expedition succeeded in collecting a total of 64,736 seeds and 1,522 metres of budwoods, mainly *H. brasiliensis*. Malaysia received 24,030 seeds or 37.5% of the collected seeds. This IRRDB project was the first major systematic attempt to replenish *Hevea*'s genetic base by collecting new germplasm from the Amazonian rainforest in a scientific manner. The germplasm materials were evaluated and are currently incorporated in the breeding programme for genetic enhancement to meet the needs of the rubber industry³.

In 1995, RRIM, Forestry Department Peninsular Malaysia (JPSM), Forest Research Institute of Malaysia (FRIM) and the Brazilian government conducted a joint expedition funded by Malaysian Timber Council (MTC) to collect seeds of various *Hevea* species mainly from the upper Amazon region in Brazil with the purpose of further increasing the genetic pool. A total of 50,231 seedlings comprising *H. brasiliensis*, *H. benthamiana*, *H. spruceana*, *H. guainensis*, *H. pauciflora*, *H. nitida*, *H. camargoana*, *H. rigidifolia* and their interspecific hybrids originating from 13 different locations in the Amazonas were successfully raised in Malaysia for evaluation and enhancement of genetic improvement⁶⁻⁸. To date, however, the genetic potential of the 1995 RRIM *Hevea* germplasm remains scarcely explored. It is therefore, crucial that evaluation of these materials be expanded in order to build new aspects of information especially on the other *Hevea* species as well as to augment the earlier selections.

The ultimate goal of this study is to form a working collection with reduced size from the germplasm for further exploitation and utilisation in the Malaysian Rubber Board (MRB) breeding programme. Results of this study will give a better insight on the genetic potential of the 1995 RRIM *Hevea* germplasm and can be used in the MRB breeding programme for the development of superior high yielding latex timber clones.

MATERIALS AND METHODS

Experimental Site and Plant Materials

The 1995 RRIM *Hevea* germplasm consists of 50,231 wild genotypes planted out in several phases in the Rantau Panjang Reserve Forest, Batu Arang, Selangor. Seedlings of the wild genotypes were

transplanted to the field from October 1995 to June 1996 and divided into two replicates with unequal sizes⁹. The initial planting distance was 2.4 m x 9.1 m with a planting density average of 447 plants/ha. The site description in terms of latitude, longitude and elevation can be denoted as 03° 24' 44" N; 111° 31' 18" E; 38 to 44 m a.s.l. Generally, the annual mean temperature and humidity for this area are 27.5°C and 84.25%, respectively. The mean annual rainfall in 2013 was 2,364.5 mm with 261 wet days while the dry season varied every year.

Phase 1 was chosen as it maintained a composition of six *Hevea* species originating from at least 10 locations in the expedition. From a total number of 5,789 genotypes occupying this area; there are 4,545 of *H. brasiliensis*, 522 of *H. spruceana*, 472 of *H. guainensis*, 111 of *H. nitida*, 101 of *H. benthamiana* and 38 of *H. pauciflora*. The breakdown of various *Hevea* species planted in Phase 1 according to their collection origins are tabulated below (Table 1).

Data Collection

At 20 years of age, each genotype was opened for tapping at 150 cm from the ground. The tapping system used was half spiral tapped once in three days (S2 d3) without stimulation. Yield was recorded based on ten tappings per tree per cycle for the duration of one year (twelve cycles) by cup-coagulation method. The genotypes which did not produce latex yield after the fourth cycle were eliminated from the tapping activities. The results were expressed in gram dry rubber per tree per tapping (g/t/t).

The truncated CBV on each genotype of different species was estimated using Trimble LaserAce 1000 rangefinder. The length, lean and volume measurement were calculated using the two points setting taken at 60 cm from the ground and at the first branching.

The following characteristics: girth at opening (GO), annual girth increment (GI) and virgin bark thickness (VB) were also

TABLE 1. MATERIALS INCLUDED IN THE STUDY AND THEIR COLLECTION ORIGINS

Collection origins	<i>Hevea</i> species						Total
	<i>H. brasiliensis</i>	<i>H. spruceana</i>	<i>H. guainensis</i>	<i>H. nitida</i>	<i>H. benthamiana</i>	<i>H. pauciflora</i>	
Acre	-	139	-	-	-	-	139
Atalaia do Norte	1,994	-	79	12	10	-	2,095
Belem	15	-	-	-	69	17	101
Benjamin Constant	447	-	-	-	-	-	447
Borba	345	52	-	-	-	-	397
Irاندوبا	43	331	-	-	-	-	374
Manaus	1,212	-	393	99	22	21	1,747
Manicore	448	-	-	-	-	-	448
Tapajos	41	-	-	-	-	-	41
Total	4,545	522	472	111	101	38	5,789

measured to complement the phenotypic evaluation on growth performance. The annual GI was computed from the annual girth measurement while VB was measured during open-tapping using a barkometer.

Selection Index

Each genotype were graded from 1 (poor) to 5 (good) for their three main characteristics which are; latex yield, clear bole volume (CBV) and girth as shown in Table 2.

Since the 1995 RRIM *Hevea* germplasm consisted of six *Hevea* species, two different parametric relationship selection indexes combining the score of latex yield, CBV and girth were adopted for the selection of potential individuals in the germplasm. Symbolically, a simultaneous parametric selection index (*I*) takes the form of Equation 1 below:

$$I = b_1X_1 + \dots + b_nX_n \quad \dots 1$$

where X_i is the observed phenotypic score of the i^{th} trait, and b_i is the weight assigned to a particular trait in the selection index¹⁰.

Therefore;

- i. The selection index for the selection of *H. brasiliensis* (I_{Hb}) are explained in Equation 2:

$$I_{Hb} = 2\text{Latex yield score} + \text{Clear bole volume score} + \text{Girth score} \quad \dots 2$$

where a value of two was assigned for the score of latex yield to indicate greater priority on the genetic potential of this trait¹¹.

- ii. As for the other *Hevea* species in the germplasm, the selection index on each species (*I*), are expressed in Equation 3:

$$I = \text{Latex yield score} + \text{Clear bole volume score} + \text{Girth score} \quad \dots 3$$

where each trait received equal weight.

In order to retain as much genetic variability as possible, 10% of the original population was proposed as a basis to determine the number of potential individuals to be selected. Selection intensity imposed on each species group was established using appropriate truncation or cut-off points. The most suitable truncation points for each species group were determined based on the desired number of individuals selected for the demand core collection.

Assessment of Major Leaf Diseases

A survey on the selected genotypes was conducted to assess the disease severity of *Oidium* and *Colletotrichum* secondary leaf fall as well as the disease incidence of

TABLE 2. SCORE CHART FOR LATEX YIELD, CLEAR BOLE VOLUME AND GIRTH AT OPENING

Score	Interval
5	$x_i > \text{Mean} + 1.25 \text{ SD}$
4	$\text{Mean} + 0.5 \text{ SD} < x_i \leq \text{Mean} + 1.25 \text{ SD}$
3	$\text{Mean} \pm 0.5 \text{ SD}$
2	$\text{Mean} - 1.25 \text{ SD} < x_i \leq \text{Mean} - 0.5 \text{ SD}$
1	$x_i \leq \text{Mean} - 1.25 \text{ SD}$

*SD: Standard deviation

Corynespora leaf disease and *Fusicoccum* leaf blight on these genotypes. Disease severity or incidence of each genotype was assessed visually by observing the extent or presence of infection on the leaves in the canopy of the tree. The severity of *Oidium* and *Colletotrichum* secondary leaf fall were scored on a scale of 0 to 3 based on the intensity of spotting for *Oidium* and spotting and deformation for *Colletotrichum* (0: no infection; 1: light infection noted on less than 10% of the canopy; 2: moderate infection noted on 10 to 40% of the canopy; 3: severe infection noted on more than 40% of the canopy) while *Corynespora* leaf disease and *Fusicoccum* leaf blight were scored based on the presence or absence of infection¹².

RESULTS

Overall Performance of Six *Hevea* Species in the 1995 RRIM *Hevea* Germplasm

The characteristics of interest specifically yield, CBV, GO, GI and VB of 5,789 genotypes in Phase 1 of the 1995 RRIM *Hevea* germplasm were measured and their variability evaluated. Potential genotypes with high latex yield, high CBV and vigorous growth were then identified and high performing genotypes were selected using the simultaneous parametric relationship selection index. The selected genotypes formed the proposed working collection.

Descriptive statistics on yield, CBV, GO, GI and VB of each *Hevea* species in the germplasm are presented in Table 3. In general, the wild genotypes conserved *ex-situ* in this germplasm produced considerably low amount of latex. The highest was recorded for *H. brasiliensis* with an average yield of 20.79 g/t/t, followed by *H. benthamiana*

(16.81 g/t/t), *H. pauciflora* (8.88 g/t/t), *H. nitida* (4.98 g/t/t), *H. spruceana* (2.82 g/t/t) and *H. guainensis* (2.67 g/t/t). Even though the latex yield was low on average, this trait possessed a high variation based on coefficient of variation values ranging from 84.01% for *H. pauciflora* to 382.98% for *H. spruceana*.

Among the genotypes of six *Hevea* species evaluated in this phase, *H. brasiliensis* performed better than that of other species in all characteristics evaluated except for GI whereby *H. pauciflora* recorded the highest value at 2.32 cm/year. Overall, *H. brasiliensis* recorded the highest mean for latex yield, CBV, GO and VB with the values of 20.79 g/t/t, 0.79 m³, 89.57 cm and 6.93 mm, respectively. On the contrary, *H. guainensis* was found to be inferior in its characteristics studied with mean latex yield of only 2.67 g/t/t, CBV of 0.25 m³, GO of 69.33 cm, GI of 1.81 cm/year and VB of 5.85 mm.

Identification of Wild Genotypes that Possess Superior Characteristics

A number of individual genotypes with outstanding performance within each species group have been identified in this phase (Figures 1 to 3). These genotypes were classified by means of ranking, using general mean and standard deviation values. Genotypes with values above the mean (+1.25 SD) could demonstrate potential in their observed characteristics.

Most of the wild genotypes recorded a low latex yield. Since the value of mean (+1.25 SD) for latex yield is low, the threshold values of 95.00 g/t/t (*H. brasiliensis*) and 25.00 g/t/t (other *Hevea* species) were fixed to increase stringency.

TABLE 3. DESCRIPTIVE STATISTICS ON MAIN CHARACTERS OF EACH SPECIES IN THE GERMPLASM

Species	Yield (g/t/t)	CBV (m ³)	GO (cm)	GI (cm)	VB (mm)
<i>H. brasiliensis</i> (n=4,454)					
Mean	20.79	0.79	89.57	2.01	6.93
Minimum	0.00	0.00	26.86	0.00	2.00
Maximum	248.01	1.84	216.00	5.60	14.00
S.E	0.46	0.01	0.44	0.01	0.02
CV (%)	149.00	62.43	32.85	29.45	24.01
<i>H. spruceana</i> (n= 522)					
Mean	2.82	0.37	81.31	1.99	6.49
Minimum	0.00	0.01	12.10	0.00	3.00
Maximum	122.82	1.19	180.00	5.10	12.00
S.E	0.47	0.01	1.09	0.03	0.06
CV (%)	382.98	64.86	30.60	36.28	20.80
<i>H. guainensis</i> (n= 472)					
Mean	2.67	0.25	69.33	1.81	5.85
Minimum	0.00	0.01	33.50	0.01	2.50
Maximum	35.92	1.10	164.40	4.90	12.50
S.E	0.23	0.01	0.90	0.03	0.07
CV (%)	183.15	76.00	28.28	31.67	26.71
<i>H. nitida</i> (n=111)					
Mean	4.98	0.35	88.27	1.94	6.83
Minimum	0.00	0.02	35.80	0.20	3.00
Maximum	64.83	0.98	157.50	5.40	13.50
S.E	0.97	0.02	2.30	0.04	0.18
CV (%)	205.42	65.71	27.40	24.13	27.74
<i>H. benthamiana</i> (n=101)					
Mean	16.81	0.40	88.37	2.03	7.15
Minimum	0.00	0.07	43.00	0.10	3.50
Maximum	81.40	0.88	148.50	5.60	13.00
S.E	2.06	0.02	2.46	0.04	0.19
CV (%)	123.14	60.00	28.00	30.24	26.25
<i>H. pauciflora</i> (n= 38)					
Mean	8.88	0.41	85.75	2.32	7.11
Minimum	0.00	0.05	37.80	0.20	4.00
Maximum	31.14	0.90	197.30	5.20	12.50
S.E	1.21	0.05	5.07	0.10	0.32
CV (%)	84.01	78.05	36.41	25.71	27.38

Yield: dry rubber in gram/tree/tapping, CBV: clear bole volume, GO: girth at opening, GI: annual girth increment, VB: virgin bark thickness, S.E: standard error, CV: coefficient of variation

Hevea brasiliensis. A total of 176 (Figure 1), 545 (Figure 2) and 497 (Figure 3) wild genotypes of this species have been identified as potential genotypes within their species group as they possess the characteristics of high latex yield, high CBV and vigorous growth, respectively. Among 176 genotypes with high latex yield, almost all were from the collection area of Manaus. Only two were from Atalaia do Norte, namely Hb/And/82/21 and Hb/And/84A/8. One was from Borba (Hb/Bb/44/1) while another was from Belem (Hb/Be/14/1). The remaining 172 genotypes were all from Manaus with the highest yield of 248.01 g/t/t (Hb/Ma/9/34), followed by 247.92 g/t/t (Hb/Ma/36/2) and 238.75 g/t/t (Hb/Ma/44/61). Genotype Hb/Bb/48A/51 recorded the highest CBV of 1.84 m³ with a straight, smooth and rounded trunk. Genotype Hb/Mc/69/1 showed the highest GO with the value of 217.00 cm.

Other Hevea Species. For identification of high latex yield, only nine wild genotypes of *H. spruceana*, six of *H. guainensis*, five of *H. nitida*, 23 of *H. benthamiana* and two of *H. pauciflora* had relatively high latex yield of more than 25.00 g/t/t (Figure 1). The highest latex yielder for *H. spruceana* was Hs/Ac/68/4 with a dry rubber yield of 122.82 g/t/t, followed by *H. benthamiana* (Hbt/Be/6A/5) with 81.40 g/t/t, *H. nitida* (Hn/Ma/19/12) with 64.83 g/t/t, *H. guainensis* (Hg/Ma/11A/55) with 35.92 g/t/t and *H. pauciflora* (Hp/Ma/51A/21) with 31.14 g/t/t.

For the identification of high CBV, only 59 individuals of *H. spruceana*, 50 of *H. guainensis*, 12 of *H. nitida*, 13 of *H. benthamiana* and four of *H. pauciflora* have been identified as high yielders within their species group in the germplasm (Figure 2). Highest CBV for *H. spruceana* was recorded

for Hs/Ac/67/39 (1.19 m³), followed by *H. guainensis* (Hg/And/72G/34; CBV = 1.10 m³), *H. nitida* (Hn/Ma/17/6; CBV = 0.98 m³), *H. pauciflora* (Hp/Be/3A/17; CBV = 0.90 m³) and *H. benthamiana* (Hbt/Be/4A/2; CBV = 0.88 m³).

Lastly, identification of wild genotypes with vigorous growth resulted in 56 individuals of *H. spruceana*, 49 of *H. guainensis*, 10 of *H. nitida*, 13 of *H. benthamiana* and 2 of *H. pauciflora* (Figure 3).

Selection of Potential Genotypes with Characteristics of Vigorous Growth and High Yield for Both Latex and Timber using Simultaneous Parametric Relationship Selection Index

Results for latex yield, CBV and GO of the selected individuals in the germplasm at different truncation points of the selection indexes are summarised in Tables 4 and 5. Overall, 616 genotypes of different species in the germplasm or 10.64% of the original population were selected to form the core collection from the 1995 RRIM *Hevea* germplasm. This core collection consists of selected genotypes with an improved performance and less variability compared to their original population.

Hevea brasiliensis. A total of 505 genotypes or 11.11% of the original population were selected at the truncation point of 13. They recorded increased average values of 86.62 g/t/t for latex yield, 1.26 m³ for CBV and 126.48 cm for GO compared to their original population in which the values were only 20.79 g/t/t, 0.79 m³ and 89.57 cm for those characteristics, respectively (Table 4). At the truncation point of 13, predicted genotypic gain from the selection for latex

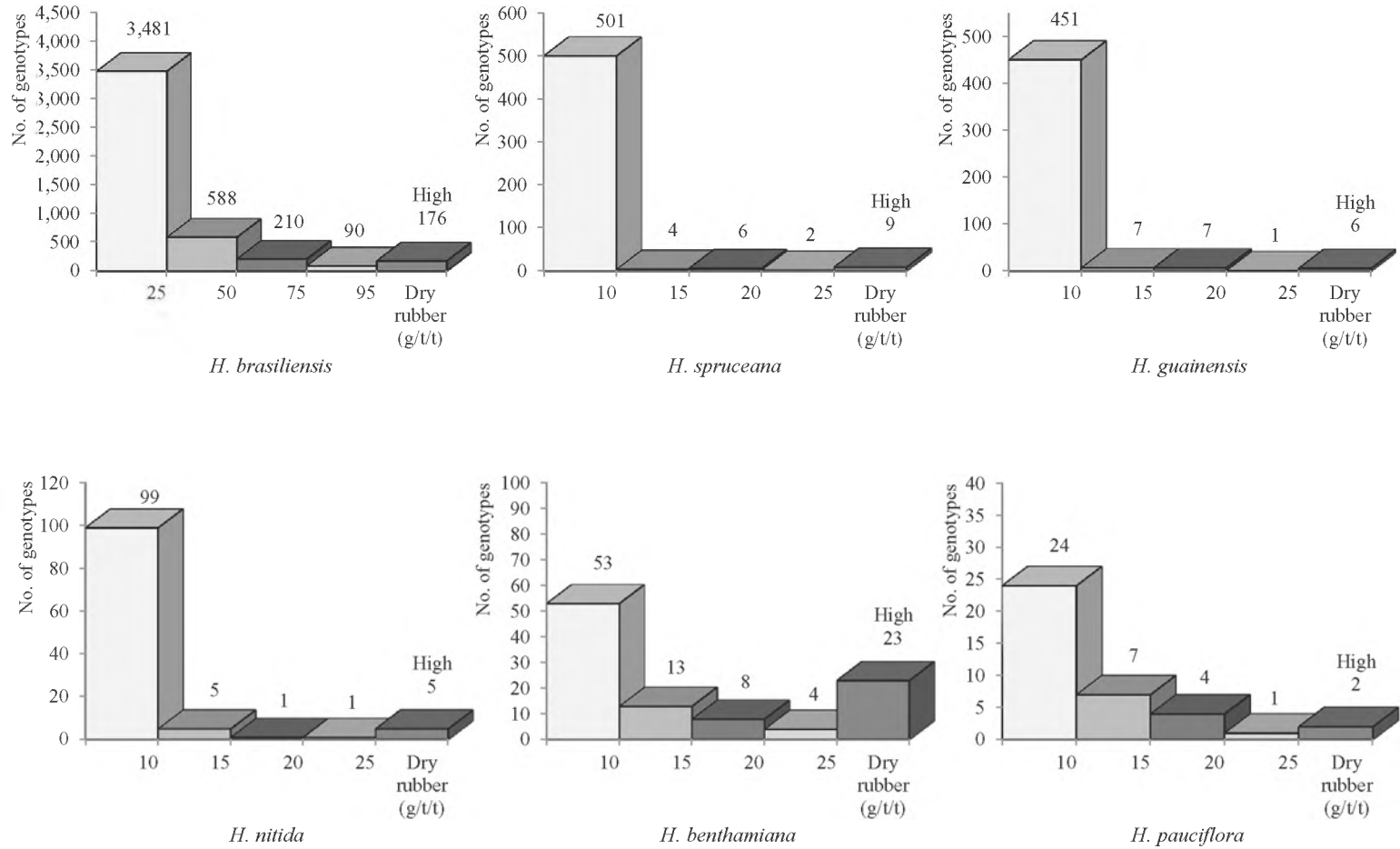


Figure 1. Distribution of latex yield for six Hevea species in the 1995 RRIM Hevea germplasm.

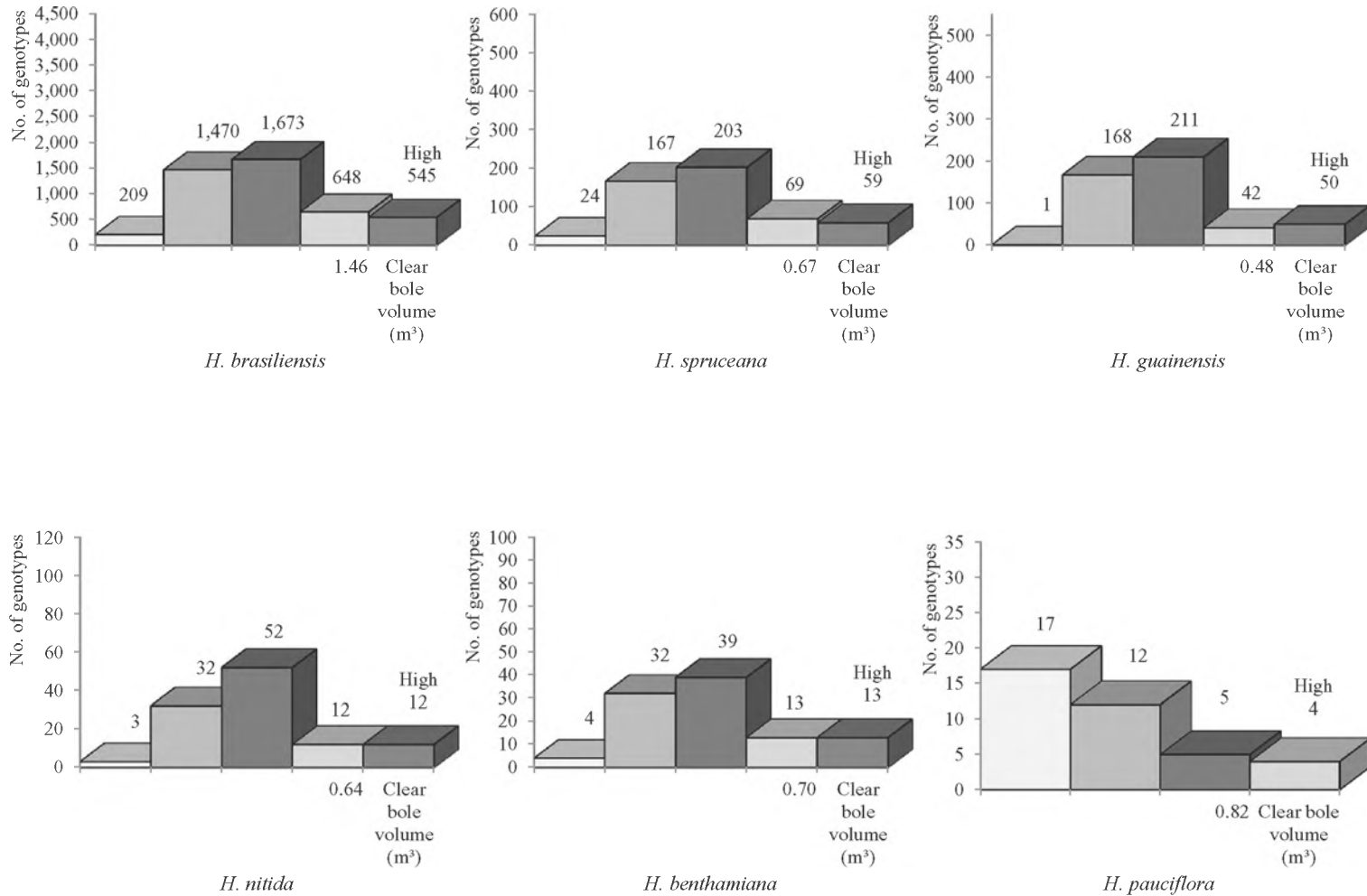


Figure 2. Distribution of clear bole volume for six Hevea species in the 1995 RRIM Hevea germplasm.

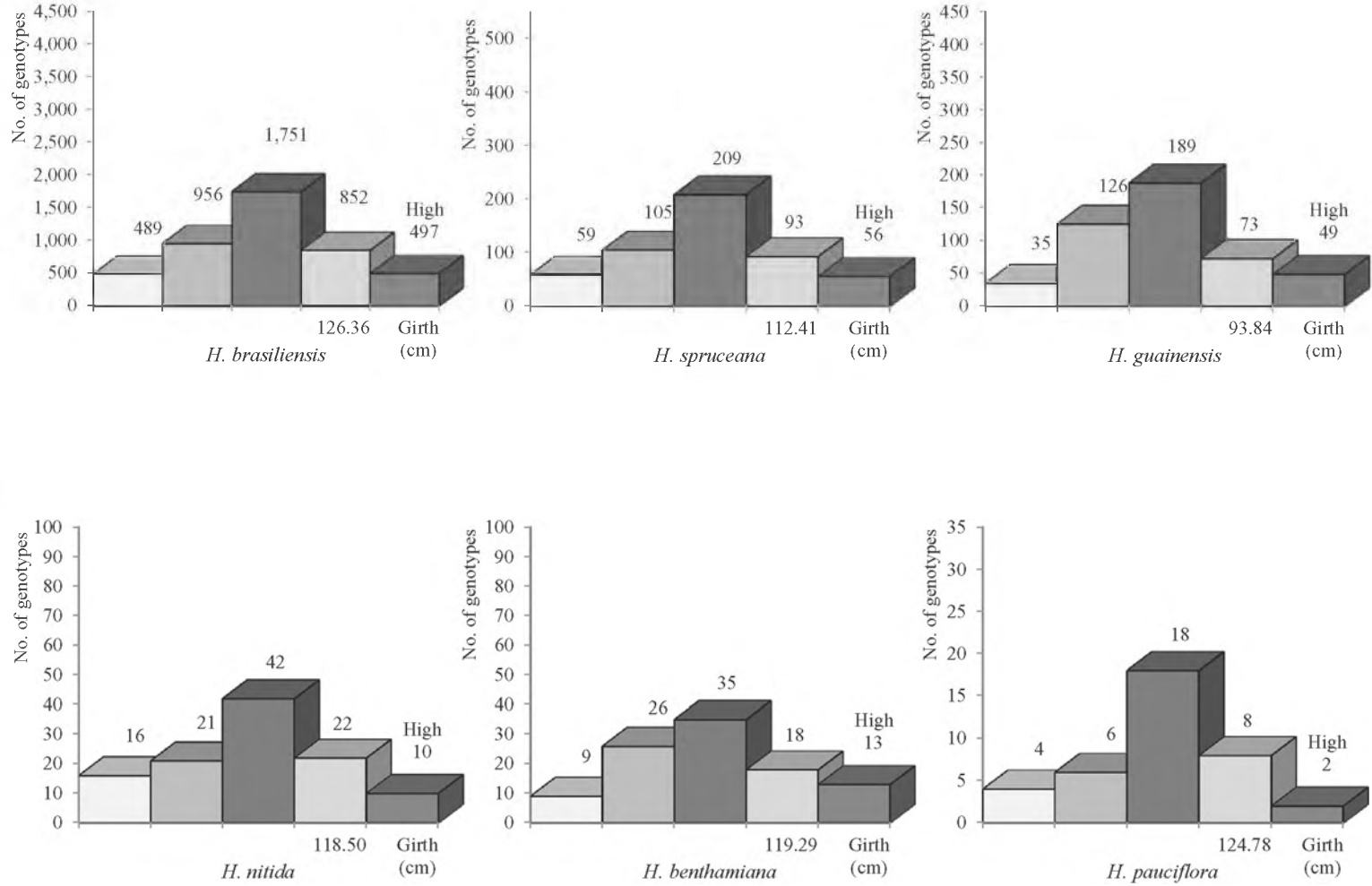


Figure 3. Distribution of girth at opening for six Hevea species in the 1995 RRIM Hevea germplasm.

TABLE 4. VALUES FOR YIELD, CLEAR BOLE VOLUME AND GIRTH AT OPENING OF THE SELECTED INDIVIDUALS OF THE 1995 RRIM *HEVEA* GERMPLASM

Species	Truncation point of the selection index	Number of genotypes selected	% original population	Yield (g/t)		CBV (m ³)		GO (cm)	
				Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
<i>H. brasiliensis</i> (n=4,454)	12	736	16.19	68.79	72.49	1.32	45.42	126.11	18.71
	13*	505	11.11	86.62	57.18	1.26	46.16	126.48	19.48
	14	399	8.78	97.28	51.32	1.27	47.51	128.38	20.11
<i>H. spruceana</i> (n=522)	11*	42	8.05	17.95	188.45	0.86	25.72	126.29	12.38
	12	11	2.10	57.14	81.33	0.80	35.26	126.75	13.72
<i>H. guainensis</i> (n=472)	10	56	11.86	7.99	130.49	0.61	36.04	103.87	17.32
	11*	38	8.05	9.00	128.32	0.64	34.26	110.69	14.56
<i>H. nitida</i> (n=111)	11*	12	10.81	20.41	116.63	0.74	40.84	124.10	14.07
	12	2	1.80	42.16	88.44	1.04	33.88	124.00	10.83
<i>H. benthamiana</i> (n=101)	12*	14	13.86	51.95	37.09	0.53	41.28	105.13	14.86
	13	5	4.95	67.55	32.77	0.72	28.82	117.60	7.53
<i>H. pauciflora</i> (n=38)	11*	5	13.16	15.80	84.43	0.82	44.91	126.90	36.65
	12	1	2.63	30.29	-	0.81	-	100.00	-

Selection index for *H. brasiliensis*: $I_{Hb} = 2 \text{ Yield} + \text{Clear bole volume} + \text{Girth}$

Selection index for other *Hevea* species: $I = \text{Yield} + \text{Clear bole volume} + \text{Girth}$

CV: Coefficient of Variation

*Selected point

(Yield, clear bole volume and girth were rated from 1 to 5)

TABLE 5. PREDICTED GENOTYPIC GAIN FROM SELECTION FOR YIELD, CLEAR BOLE VOLUME AND GIRTH AT OPENING OF *H. BRASILIENSIS* IN THE GERMPLASM

Truncation point of the selection index	Yield			CBV			GO		
	Differential (g/t/t)	Genotypic gain (g/t/t) %		Differential (m ³)	Genotypic gain (m ³) %		Differential (cm)	Genotypic gain (cm) %	
12	48.00	46.56	223.95	0.53	0.28	35.56	36.54	24.85	27.74
13*	65.83	63.86	307.14	0.47	0.25	31.53	36.91	25.10	28.02
14	76.49	74.20	356.88	0.48	0.25	32.20	38.81	26.39	29.46
Broad-sense heritability		0.97			0.53			0.68	

Mean of original population for yield, clear bole volume and girth at opening of *H. brasiliensis* were 20.79 g/t/t, 0.79 m³ and 89.57 cm, respectively.
g/t/t: gram dry rubber/tree/tapping

*Selected point

yield, CBV and GO was equal to 307.14%, 31.53% and 28.02%, respectively (Table 5).

Other Hevea Species. A total of 14 potential genotypes of *H. benthamiana* (13.86%) were selected at truncation point of 12 while 42 of *H. spruceana* (8.05%), 38 of *H. guainensis* (8.05%), 12 of *H. nitida* (10.81%) and five of *H. pauciflora* (13.16%) were selected at a lower truncation point of 11 (Table 4).

Assessment of Major Leaf Diseases on the Selected Genotypes

The incidence of *Oidium* and *Colletotrichum* secondary leaf fall as well as *Corynespora* leaf disease and *Fusicoccum* leaf blight were assessed to determine the susceptibility of the selected genotypes to the aforementioned major leaf diseases. Overall, none of the selected genotypes were severely infected by any of the diseases. Very few of them were moderately infected by *Oidium* and *Colletotrichum* secondary leaf fall while most of them exhibited light infection or no infection at all.

Only 9.09% of *H. spruceana* and 14.79% of *H. brasiliensis* were moderately affected by *Oidium* secondary leaf fall. The majority of *H. benthamiana* (71.43%), *H. nitida* (83.33%) and all the 38 selected *H. guainensis* (100%) were found to be free from this disease. Additionally, 81.82% of *H. spruceana*, 77.92% of *H. brasiliensis* and 60% of *H. pauciflora* were found to be lightly infected (Figure 4).

Genotypes of all six *Hevea* species were largely free from *Colletotrichum* secondary leaf fall. Only 20% of *H. pauciflora* and 1.10% of *H. brasiliensis* were moderately affected by this disease. 21.43% of *H. benthamiana*, 16.67% of *H. nitida*, 20% of *H. guainensis*,

54.55% of *H. spruceana* and 32.89% of *H. brasiliensis* were recorded to have light infection (Figure 5). None of the genotypes assessed were affected by *Corynespora* leaf disease or *Fusicoccum* leaf blight.

DISCUSSION

Prior to the selection process, the variability of the main characteristics of *Hevea* species in the 1995 RRIM *Hevea* germplasm was first examined and potential genotypes which possess desired characteristics were then identified. In general, the latex yield of the wild genotypes in this germplasm can be considered poor with the average latex yield ranging from only 2.67 g/t for *H. guainensis* to 20.79 g/t for *H. brasiliensis*. Only 19.16% of these germplasm materials were found to produce an acceptable amount of latex of more than 25.00 g/t.

In the previous work on the 1981 IRRDB *Hevea* germplasm collection, it was reported that the materials exhibited very poor performance with an average latex yield amounting 16% of the level for the currently developed Wickham clones after five years of tapping in Vietnam¹³. In Malaysia, it was reported that non-stimulated yield means ranged from 1.80 to 13.86 g/t for seedlings derived from three different states of the 1981 IRRDB *Hevea* germplasm at the 8th year of tapping¹¹.

With the exception of *H. pauciflora*, latex yield was found to have a wide variability as indicated by the high coefficient of variation of over 100%. This variation is considered high even when compared to Wickham seedlings which yield typically between 25% and 55%¹⁵. A similar study was conducted on several *Hevea* species from the 1966 importation¹⁶. The variation

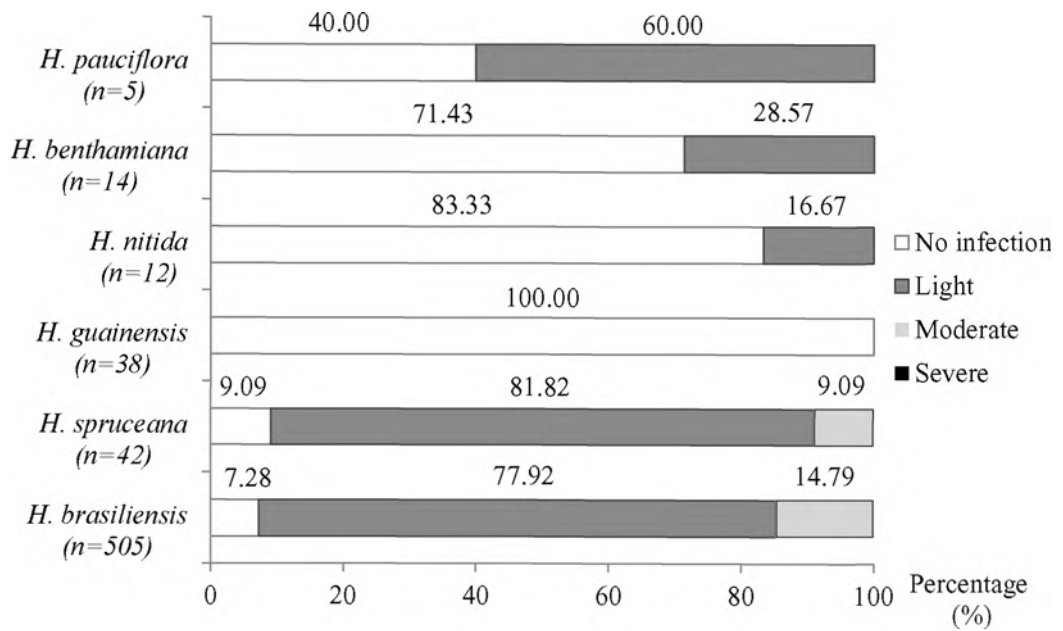


Figure 4. Incidence of *Oidium* secondary leaf fall on selected genotypes of six *Hevea* species based severity of infection.

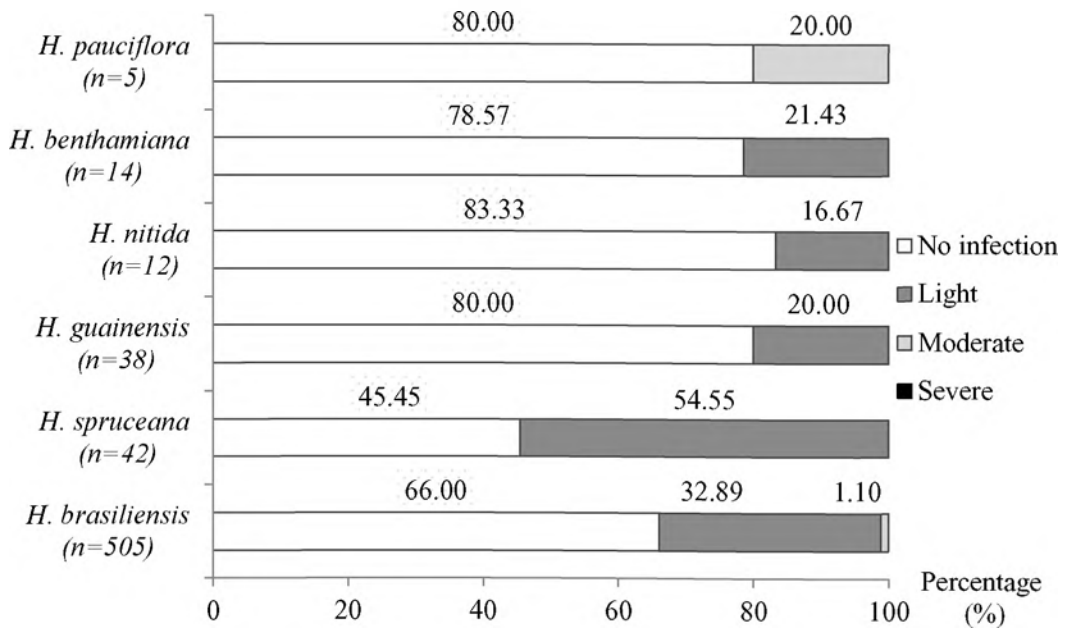


Figure 5. Incidence of *Colletotrichum* secondary leaf fall on selected genotypes of six *Hevea* species based severity of infection.

values for yield on the first year of tapping were found to be 41.84% for *H. pauciflora* var. *coriacea*, 36.41% for *H. nitida*, 31.95% for *H. guainensis*, 22.00% for *H. pauciflora*, 16.33% for *H. benthamiana* and 12.12% for *H. pauciflora*. The low variation values might be due to a small sample size of only 76 surviving genotypes involved in the experiment.

Although most of the wild genotypes were low latex yielders, several individuals with exceptional characteristics within each species group were identified. For identification of high latex yield, 176 wild genotypes of *H. brasiliensis* were found to yield latex exceeding the threshold value of 95.00 g/t when tapped at the age of 20 years. Latex yield is affected by climatic factors, soil nutrients, diseases, tree age, competition among and between species as well as other agronomic factors^{17,18}. Thus, the chances for direct application of these materials for commercial planting are probably impractical. These materials should be multiplied and further tested on a proper experimental scale. Systematic evaluation of these genetic materials will enable selection of potential genotypes to be incorporated into breeding programmes for development of superior latex and timber clones.

For identification of high estimated CBV, 545 of *H. brasiliensis*, 59 of *H. spruceana*, 50 of *H. guainensis*, 12 of *H. nitida*, 13 of *H. benthamiana* and four of *H. pauciflora* were categorised as high yielders within their species group in the germplasm. Lastly, 497 of *H. brasiliensis*, 56 of *H. spruceana*, 49 of *H. guainensis*, 10 of *H. nitida*, 13 of *H. benthamiana* and two of *H. pauciflora* were identified to have vigorous growth.

A similar study searching to identify potential genotypes for timber production in Malaysia reported that among the 24 year old

clones from six *Hevea* species evaluated in the 1966 germplasm collection, *H. guianensis* (HHB 20A/5) had the highest CBV and total wood volume of 1.77 m³ and 4.43 m³ per tree, respectively. Others with good CBV were *H. guainensis* with 1.14 m³ per tree (HHB 20A/3), *H. pauciflora* with 1.50 m³ per tree (HHB 10/1) and *H. nitida* with 1.08 m³ per tree (HHB 7/1)¹⁹.

The fast growing genotypes with long clear boles have the potential to be used for establishing plantations which are meant for timber production. Intra and interspecific crosses among the selected genotypes and the present fast growing clones can be carried out to produce progenies for selection. Succeeding several testing and selection stages, the progenies can be recommended to rubber forest plantations in order to meet rubberwood demand from timber based industries.

The 1995 RRIM *Hevea* germplasm can be utilised to broaden the *Hevea* genetic base and enhance the *Hevea* breeding programme at Malaysian Rubber Board (MRB). However, it is impossible to work with the whole collection, owing to shortage of resources and facilities. Therefore, establishing a working collection of reduced size containing only selected genotypes is a practical solution. Selection of multiple characteristics of wild genotypes in this study was carried out using simultaneous parametric relationship selection index¹⁰.

In the selection process of wild germplasm for any crop improvement programme, the goal is to gather good performance genotypes as well as to retain as much genetic variability possible. Thus, the selection intensity imposed on the original population should be appropriate. For *H. brasiliensis*, if the truncation point of the selection index of 13 was chosen, the working collection

of reduced size would be 11.11% of the original population, or 505 genotypes, while maintaining a considerably high variation for yield and the estimated CBV.

Ten percent of the parent collection is generally accepted as core collection. This proportion, in theory, should retain more than 70% of the alleles in the parent collection. However, for a very large collection, a core collection of 10% might still amount to a very large number of accessions to deal with. Therefore, 3,000 as an upper limit for the size of a core collection was suggested²⁰. The reduced collection size will be both cost effective and easier to maintain.

The predicted genotypic gain from the selection of *H. brasiliensis* was highest for latex yield, followed by the estimated CBV and GO at the truncation point of 13. For other *Hevea* species in the germplasm, a total of 42 potential genotypes of *H. spruceana* (8.05%), 38 of *H. guainensis* (8.05%), 14 of *H. benthamiana*, 12 of *H. nitida* (10.81%) and five of *H. pauciflora* (13.16%) were selected from their species group. Overall, the selected genotypes amounted to a total number of 616 genotypes of six species or 10.64% of the original population.

Assessment of major leaf diseases on the selected genotypes revealed that most of the genotypes were free from or only lightly infected by *Oidium* and *Colletotrichum* secondary leaf fall. No incidence of *Corynespora* leaf disease and *Fusicoccum* leaf blight were recorded.

Differences in the levels of resistance to important diseases have been observed in 1981 IRRDB germplasm collections from different countries. In India, a large number of these accessions were shown to be resistant to *Oidium* secondary leaf fall (140 accessions) and *Corynespora* leaf

disease (70 accessions)²¹. Similarly, a total of 21 accessions in 1981 IRRDB germplasm collection were resistant to *Oidium* secondary leaf fall in a bush-wood garden in China²². Resistance to South American Leaf Blight (SALB) was observed in 298 accessions from germplasm collection planted in both the French Guyana and Brazil, of which the accessions from Acre and Rondonia were most resistant²³. These results have contributed to the development of clonal disease resistance by genetic recombination using 1981 IRRDB germplasm collection as paternal clones in the *Hevea* breeding programmes at several countries.

Utilisation of several *Hevea* species for the development of disease resistant clones particularly resistance towards SALB have been reported. *H. benthamiana* and *H. pauciflora* were used in breeding programmes for their high resistance level towards SALB, particularly *H. benthamiana* F 4542 (Ford 4542). *H. guainensis*, *H. microphylla* and *H. spruceana* have been crossed with *H. brasiliensis* to produce interspecific hybrids²⁴.

A genetic analysis of clones and progenies observed in the field and exposed to *Colletotrichum*, *Corynespora* and *Oidium* concluded that selection against these leaf diseases can be integrated in the general programme of breeding for rubber yield and desirable secondary traits²⁵.

However, no exact conclusion on disease resistance can be drawn from this assessment alone because the observations were made under natural conditions and disease infections were rather mild during the observation period. Accordingly, laboratory assay and artificial inoculation of the pathogen in the field can be conducted in order to further evaluate the susceptibility or resistance of the selected genotypes to major rubber diseases.

CONCLUSIONS

Based on this evaluation, a working collection of 505 potential genotypes of *H. brasiliensis*, 42 of *H. spruceana*, 38 of *H. guainensis*, 14 of *H. benthamiana*, 12 of *H. nitida* and five of *H. pauciflora* was formed for further studies. This may include the evaluation of these genotypes on physiological and anatomical characteristics, properties of rubber, timber quality and disease resistance under undesirable environmental conditions. Since the assessment of major leaf diseases on the selected genotypes revealed that most of the genotypes were free from or only lightly infected by *Oidium* and *Colletotrichum* secondary leaf fall, further evaluation using laboratory assay and artificial inoculation of the pathogen in the field have to be conducted in order to confirm the susceptibility or resistance of the selected genotypes to major leaf diseases. This study gave a better understanding on genetic potential of the 1995 RRIM *Hevea* germplasm which can help in planning breeding programmes and utilisation of this germplasm in Malaysia. The results can also serve as reference information for *Hevea* germplasm improvement programmes in other countries.

ACKNOWLEDGEMENTS

The authors would like to thank the Malaysian Rubber Board for financial assistance. Special thanks to Dr Zairossani Mohd Nor, the Director General of MRB and Dr Mohd Nasaruddin Mohd Aris, Director of Production Development Division for their comments and approval of this paper for publication. Special appreciation goes to the following persons for their field assistance and data compilation, Norshahida Sharifudin, Mohd Zairil Yusof and Ramakrisnan a/l Ellapan. Lastly, the authors are also indebted to Dr Ramli Othman, Fellow of International

Rubber Research and Development Board, for his encouragement, comments and criticisms.

Date of receipt: February 2017

Date of acceptance: June 2017

REFERENCES

1. MALAYSIAN RUBBER BOARD (2009) Rubber Plantation and Processing Technologies. Kuala Lumpur: MRB.
2. MALAYSIAN RUBBER BOARD (2016) Natural Rubber Statistics 2016. Kuala Lumpur: MRB.
3. ONG, S.H., MOHD NOOR, A.G., TAN, A.M., AND TAN, H. (1983) New *Hevea* Germplasm: Its Introduction and Potential. *Proc. RRIM Planters Conf.*, Oct 17 – 19, 1983, Kuala Lumpur: RRIM, 3 – 17.
4. ONG, S.H., RAMLI, O., OTHMAN, H., MASAHULING, B., AND NAIMAH, I. (1994) Rubber Breeding: Progress and Strategies to Meet Future Needs of the Plantation Industry. In Management for Enhanced Profitability in Plantations. Chee, K.H (ed.) Kuala Lumpur: Incorporated Society of Planters, 53 – 70.
5. TAN, H. (1987) Strategies in Rubber Tree Breeding. In: Improving Vegetatively Propagated Crops. Abbott, A.J. and Atkin, R.K. (eds.) London: Academic Press, 27 – 62.
6. MASAHULING, B. AND RAMLI, O. (1995) Introduction and *ex-situ* Conservation of *Hevea* Genetic Resources. *Int. Symp. Workshop on Conservation Biol.*, Nov. 19 – 23, 1995, Kuching, Sarawak.
7. RUBBER RESEARCH INSTITUTE OF MALAYSIA (1997) Rubber Research Institute Malaysia Annual Report 1997. Kuala Lumpur : RRIM.

8. MALAYSIAN RUBBER BOARD (1999) Malaysian Rubber Board Annual Report 1999. Kuala Lumpur: MRB.
9. MOHD ARIS, M.N.B., MASAHULING, B., AND RAMLI, O. (2002) Progress Report of 1995 Hevea Germplasm Introduction to Year 2002. Kuala Lumpur : MRB.
10. BAKER, R.J. (1986) Selection Indices in Plant Breeding. Florida: CRC Press.
11. RRIM PLANTING RECOMMENDATIONS COMMITTEE (1995) RRIM Planting Recommendations 1995-1997. *Planters' Bull.* **224-225(1)**, 51 – 72.
12. TAN, A.M., LOO, T.P., VADIVEL, G., BACHIK, M.R. AND YOON, K.F. (1992) Survey of Major Leaf Diseases of Rubber in Peninsular Malaysia. *Planters' Bull.* **211**, 51 – 62.
13. VAN, L., THANH, T., THUY TRANG, L. T., VAN, V., BAO, H. AND MAU, L. (2012) *Hevea* Germplasm in Vietnam: Conservation, Characterization, Evaluation and Utilisation. Genetic Diversity in Plants. In: Genetic Diversity in Plants. Caliskan, M. (ed.) Croatia: Intech, 433 – 456.
14. MASAHULING, B., RAMLI, O. AND ZARAWI, A. G. (2005) Progress in Designing Future Clones Through Utilisation of New Germplasm. *Proc. Rubb. Planters' Conf. 2005*, 15 – 17 November, Kuala Lumpur, Malaysia. Kuala Lumpur: MRB.
15. DIJKMAN, M.J. (1951) Hevea: Thirty Years of Research in the Far East. Florida: University of Miami Press.
16. ONG, S.H. AND TAN, H. (1987) Utilisation of Hevea Genetic Resources in the RRIM. *Malays. Appl. Biol.*, **16(1)**, 145–155.
17. LI, G.Y., WANG, Q.B. AND LI, Y.Y. (2013) A Review of Influencing Factors on Latex Yield of *Hevea brasiliensis*. *Chin. J. Ecol.* **32(10)**, 510 – 517.
18. RODRIGO, V.H.L. (2007). Ecophysiological Factors Underpinning Productivity of *Hevea brasiliensis*. *Braz. J. Plant Physiol.* **19(4)**, 245 – 255.
19. RAMLI, O., ARSHGAD, N.L., HUAT, O.S., HASHIM, O., MASAHULING, B., WANCHIK, M.G., AZIZ, M.Z.A., GHANI, Z.A. AND GHANI, M.N.A. (1995) Potential *Hevea* Genotypes for Timber Production. *Proc. Rubb. Growers' Conf.*, Jul. 17-19, 1995, Kuala Lumpur Malaysia. Kuala Lumpur: MRB, 340 – 360.
20. UPADHYAYA, H.D. AND GOWDA, C.L.L. (2009) Managing and Enhancing the Use of Germplasm – Strategies and Methodologies. Monograph. Hyderabad, India: International Crops Research Institute for the Semi-Arid Tropics.
21. VARGHESE, Y.A., ABRAHAM, S.T., MERCY, M.A., MADHAVAN, J., REGHU, C.P., RAO, G.P., AMMAL, S.L., IDICULA, S.P. AND JOSEPH, A. (2002) Management of the 1981 Germplasm Collection in India, *IRRDB Joint Workshop on Breeding, Agronomy and Socioeconomy*, Aug 28 – Sep 7, 2002, Malaysia & Indonesia. Kuala Lumpur: IRRDB.
22. HUANG, H., YU, D. AND ZHOU, J. (2002) Studies of the 1981 IRRDB *Hevea* germplasm in China. *IRRDB Joint Workshop on Breeding, Agronomy and Socioeconomy*, Aug 28 – Sep 7, 2002, Malaysia & Indonesia. Kuala Lumpur: IRRDB.
23. LE GUEN, V., GARCIA, D., MATTOS, C. R. R. AND CLEMENT-DEMANGE, A. (2002) Evaluation of Field Resistance to *Microcyclus ulei* of a Collection of Amazonian Rubber Tree (*Hevea brasiliensis*) Germplasm. *Crop Breed. Appl. Biotechnol.* **2(1)**, 141 – 148.

24. NARAYANAN, C. AND MYDIN, K.K. (2012) Breeding for Disease Resistance in *Hevea* spp. - Status, Potential Threats, and Possible Strategies. In: *Proc. Fourth Int. Workshop on the Genetics of Host-parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees*. Sniezko, A.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M. and Frankel, S.J. (eds.) California: U.S. Department of Agriculture, 240 – 251.
25. TAN, H. AND TAN, A.M. (1996) Genetic Studies of Leaf Disease Resistance in *Hevea*. *J. nat. Rubb. Res.* **11(1)**, 108 – 114.