Management of the 1981 IRRDB Germplasm Collection in India

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Abstract

Rubber cultivation in India has been extended to marginal and non-traditional areas confronted with various agro climatic constraints limiting plant growth and productivity. Hence, development of location specific clones capable of withstanding specific stress situations has become a priority area of research. In this context, the wild germplasm collected from the three provenances in Brazil viz. Acre (AC), Rondonia (RO) and Mato Grosso(MT) is a valuable raw material for achieving this objective and thereby gaining further genetic advance in crop improvement programmes.

In India, currently, a total of 4548 wild *Hevea* germplasm accessions imported from the Malaysian center during the period 1984-1990 are being conserved. Systematic efforts are underway for conservation, characterization, evaluation and documentation of these valuable genetic resources aimed at their effective utilization for achieving sustainable yield levels.

1. Introduction

The Rubber Research Institute of India has been making efforts on conservation, evaluation and utilization of the genetic resources of *Hevea* with main emphasis on wild germplasm resultant of the 1981 IRRDB-EMBRAPA expedition to the center of diversity in Brazil. Since the original gene pool of *Hevea brasiliensis* utilized for the development of popular clones is narrow, greater genetic variability is essential for further genetic advance and attaining stability in yield through incorporation of resistance to various biotic and abiotic stresses limiting crop productivity. Natural genetic variability is a useful source of individual alleles that confer adaptive advantages such as resistance to diseases and pests, drought etc. Thus the wild *Hevea* genotypes are a potential source for introgression of certain oligogenic as well as polygenic characters complementing the present day cultivated clones.

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2. Conservation

In India, *Hevea* genetic resources have been established and maintained in conservation nurseries and gardens in the traditional and / or non-traditional areas. The wild germplasm is being conserved in source bush nurseries (Table 1), at a spacing of 1 x 1m, with each genotype represented by 5-10 plants along with selected popular Wickham clones as controls. In the conservation nurseries, one plant per genotype was allowed to grow without regular pruning in order to gather early information on tree habit, flowering pattern, floral characteristics and seed morphology.

Table 1: Details of the conservation nurseries

Year	Identity of Source Bush Nursery	No of accessions	Location
Conserved in the traditional region			
1984	Source Bush Nursery – A	100	Central Experiment Station, RRII
1985	Source Bush Nursery – B	83	Central Experiment Station,RRII
1988	Source Bush Nursery – C	873	Central Experiment Station,RRII
1989	Source Bush Nursery – D1	144	Central Experiment Station,RRII
1989	Source Bush Nursery – D2	1623	Central Experiment Station,RRII
1991	Source Bush Nursery – E	560	Central Experiment Station,RRII
1991	Source Bush Nursery – F	193	Central Experiment Station,RRII
2000*	Source Bush Nursery – G	199	Main Campus, RRII, Kottayam
2001*	Source Bush Nursery – H	58	Central Experiment Station,RRII
2002*	Source Bush Nursery - I	86	Regional Station, Padiyoor,RRII
2002*	Source Bush Nursery - J	56	Regional Station, Padiyoor,RRII
Total		**3975**	
Conserved in the non traditional region			
1987,1988	Source Bush Nursery	587	Regional Station, Guwahati,N.East
1987,1990	Source Bush Nursery	385	Regional Station, Agartala, N.East
Total		**972**	

* Available in the N.East conservation nurseries also

Table 2. Provenance and locality wise details of conservation

Provenance	Localities in Brazil (No.)	Present status – No. conserved in		Total
		Kerala	North East	
Acre	5	1530	242	1772
Rondonia	7	1540	348	1888
Mato Grosso	4	465	382	847
Others		41		41
Total	16	3576	972	4548

3. Characterisation and documentation

3.1. Morphological characterization

With the objective of characterization of the wild germplasm, a descriptor has been prepared which includes passport data, plant type description at juvenile phase and data on relevant quantitative and qualitative characters expressed in subsequent phases of growth (Abraham *et al.*, 1994). Based on morphological characterization in the field trials in the juvenile phase, 438 accessions have been catalogued in Table 3.

Table 3 Cataloguing of a group of wild accessions based on morphological descriptors.

Sl. No	Character	AC	RO	MT	Sl. No	Character	AC	RO	MT
1	**Height of the plants**				6.2.3.2	Perpendicular	102	132	41
1.1	Dwarf	38	64	20	6.2.3.3	Obtuse	1	0	0
1.2	Medium tall	110	104	52	**6.3**	**Petiolule**			
1.3	Tall	14	25	11	**6.3.1**	**Orientation**			
2	**Girth of the plants**				6.3.1.1	Upward	52	60	32
2.1	Below average	37	42	14	6.3.1.2	Horizontal	76	99	41
2.2	Average	112	133	51	6.3.1.3	Downward	34	34	10
2.3	Above average	13	18	18	**6.4**	**Leaflets**			
3	**Nodes**				**6.4.1**	**Colour**			
3.1	**Axillary buds**				6.4.1.1	Green	140	160	63
3.1.1	Protruding	8	3	2	6.4.1.2	Dark green	20	20	17
3.1.2	Sunken	6	15	2	6.4.1.3	Yellowish green	2	12	3
3.1.3	Normal	148	175	79	**6.4.2**	**Lustre**			
3.2	**Leaf scars**				6.4.2.1	Glossy	9	25	24
3.2.1	Pronounced margin	54	52	17	6.4.2.2	Dull	153	168	59
3.2.2	Normal margin	108	141	66	**6.4.3**	**Texture**			
4	**Leaf storey**				6.4.3.1	Leathery	42	55	40
4.1	Shape				6.4.3.2	Normal	120	138	43
4.1.1	Conical	3	2	1	**6.4.4**	**Shape**			
4.1.2	Truncate	28	34	13	6.4.4.1	Elliptic	122	160	72
4.1.3	Bow shaped	40	39	15	6.4.4.2	Lanceolate	28	16	5
4.1.4	Hemispherical	91	118	54	6.4.4.3	Obovate	12	17	6
5.2	**Separation**				**6.4.5**	**Margin**			
5.2.1	Well separated	127	131	61	6.4.5.1	Entire	121	130	55
5.2.2	Not well separated	12	25	4	6.4.5.2	Wavy	41	63	28
5.2.3	Intermediate	23	36	18	**6.4.6**	**Size**			
5.3	**External appearance**				6.4.6.1	Large	28	28	9
5.3.1	Open	147	170	77	6.4.6.2	Medium	125	150	68
5.3.2	Close	15	22	6	6.4.6.3	Small	9	15	6
6	**Leaves**				**6.4.7**	**Appearance** (Cross section)			
6.1	**Pulvinus**				6.4.7.1	Straight	42	79	42
6.1.1	Swollen	67	69	38	6.4.7.2	V' shaped	12	16	11
6.1.2	Normal	95	124	45	6.4.7.3	Boat shaped	108	98	30
6.2	**Petiole**				**6.4.8**	**Leaf apex**			
6.2.1	**Shape**				6.4.8.1	Aristate	16	25	8
6.2.1.1	Arched	8	13	6	6.4.8.2	Cuspidate	42	73	29
6.2.1.2	Concave	40	41	14	6.4.8.3	Apiculate	104	95	46
6.2.1.3	Straight	90	118	55	6.4.8.4				
6.2.1.4	S' shape	24	21	8	**6.4.9**	**Vein colour**			
6.2.2	**Size**				6.4.9.1	Yellow	42	50	38
6.2.2.1	Short	24	37	14	6.4.9.2	Light yellow	120	143	45
6.2.2.2	Medium	109	141	61					
6.2.2.3	Long	29	15	8	**6.4.10**	**Leaf blade dorsal side**			
6.2.3	**Angle**				6.4.10.1	Flat	131	163	69
6.2.3.1	Acute	59	61	42	6.4.10.2	Irregular	31	30	14

Predominant characters include plants with medium girth and height, normal axillary buds and leaf scars, hemispherical/bow shaped, well separated and open canopy, straight medium sized perpendicularly placed petioles, leaflets medium sized, green elliptic leaves with entire margin, apiculate apex.

3.2. Molecular characterization

Molecular markers being very useful in managing the large germplasm collection, studies have been initiated for cataloguing the genetic diversity among genotypes from different sources. Characterization at the molecular level helps to eliminate the effect of the environment and to provide precise information on the extent and distribution of genetic diversity among genetic resources.

Genomic characterization using PCR based DNA markers have been initiated with the objective of documenting DNA profiles and identification of possible duplicates. RAPD assay has proved to be the fastest, most cost effective and efficient method for molecular characterization and assay of genetic diversity in the large collection of genotypes (Varghese *et al,*1997, 2000). RAPD profiling incorporating 143 wild accessions using 20 informative primers is in progress. The profiles indicate considerable genetic variation among the genotypes from Acre, Rondonia and Mato Grosso provenances (Varghese *et al*, 2002). Another study involving 60 wild accessions using 22 primers could discriminate the wild genotypes based on their geographic location through unique amplification profiles (RRII, 2000).

4. Documentation

Being the national agency dealing with *Hevea* research, the Rubber Research Institute of India maintains a National Accession Register with all basic data on the wild germplasm. Recently a user-friendly software has been developed for documentation of the detailed database of *Hevea* germplasm, including passport and collection details, details of conservation nurseries and evaluation trials and plant data generated in the different phases of evaluation, for storage and easy retrieval as and when necessary. A herbarium collection of the wild accessions is being prepared and maintained. Floral, fruit and seed variability is also being documented.

5. Evaluation

Field evaluation of the wild germplasm is done in statistically laid out trials in two stages viz.a preliminary evaluation, where the accessions are evaluated for agro-morphological and anatomical characters, and a further evaluation, where selections from the preliminary evaluations are subjected to a more detailed study.

5.1. Preliminary evaluation

The wild accessions are evaluated in a phased manner in Preliminary Evaluation Trials (PET) for studying their performance under field conditions for various agro-morphological and anatomical characters. The preliminary evaluation trials are laid out employing augmented randomized block design (RBD) or simple lattice design with suitable controls, so as to accommodate a maximum number of genotypes at a time. The ortets, being preliminary selections and few in number, are evaluated in an RBD or lattice design. So far a total of 1253 accessions have been established in 16 preliminary evaluation trials in different stations in the traditional and non-traditional rubber growing areas.

Table 4. Details of the Preliminary Evaluation Trials (PETs)

Trial	Year	Location	Accessions	Statistical design	Replications	Plot size	Controls
1	1990	CES, Chethackal*	338	Augmented CRD	-	5	RRII 105
2	1992	CES, Chethackal*	80	Simple lattice	4	4	RRII 105
3	1992	CES, Chethackal *	63	Simple lattice	4	4	RRII 105
4	1994	CES, Chethackal*	24	Simple lattice	4	4	RRII 105
5	1994	CES, Chethackal*	24	Simple lattice	4	4	RRII 105
6	1994	CES, Chethackal*	24	Simple lattice	4	4	RRII 105
7	1999	CES, Chethackal*	46	Simple lattice	4	4	RRII105, PB260, RRIM600
8	2000	RRS, Padiyoor*	168	Augmented RBD	-	5	RRII105, RRII208, RRIM 600
9	2000	RRS, Padiyoor*	1 66	Augmented RBD	-	5	RRII105 RRII203. RRIM 600
10	2000	RRII, Kottayam*	47	Augmented RBD	-	5	RRII105, RRIM 600
11	2000	RES, Nagrakatta**	46	Simple lattice	4	4	RRII105, SCATC93-112,RRIM600
12	2000	RES, Nagrakatta**	18	RBD	3	5	RRIM600, PB 235, Haiken1. RRII 203

13	2001	RRS, Dapchari**	63	Augmented RBD	-	5	RRII105, RRIM600, Tjir1
14	2002	RRS, Dapchari**	42	Augmented RBD	-	5	RRII105, RRIM600, Tjir1
15	2002	RRS, Padiyoor*	48	Augmented RBD	-	5	RRII105, RRII208, RRIM 600
16	2002	RRS, Padiyoor*	56	Augmented RBD	-	5	RRII105, RRIM 600

* Traditional rubber growing area ** Non traditional rubber growing area

Data so far have revealed wide variability and significant genetic differences among the genotypes for most of the agro morphologic traits, bark structural characters and juvenile yield indicating the scope for selection of accessions with desirable characters (Annamma *et al* 1989; Abraham *et al* 1992,2000; Madhavan *et al* 1993; Mercy *et al* 1993, 1995, Rao *et al* 1996). Out of 553 accessions in five PETs, 25 % recorded superior growth in terms of girth. There were several individual genotypes with values higher than that of the control clone for several growth characters like girth, height, number of leaves, number of leaf whorls etc (Abraham *et al* 2000) and bark anatomical characters like bark thickness, number of latex vessel rows, density and diameter of latex vessels (Reghu *et al* 1996). Studies so far have revealed that several accessions were superior for individual traits in comparison to the control clone RRII 105. These include 125 for girth, 29 for total number of latex vessel rows, 41 for bark thickness and 15 accessions with relatively high yield in the immature phase. In general Acre and Rondonian genotypes were more vigorous in growth than those from Mato Grosso provenance. As expected, the wild accessions were low yielders compared to the popular Wickham clones. However, a few individual accessions with comparable or even higher yield than the popular clone RRII 105 have been identified in the early growth phase. Out of the first three trials in tapping stage including 480 accessions, relatively good yield trend with 50 –65 % of the yield of the control was recorded in 8 accessions with two of them on par with the control Wickham clone RRII 105. Accessions from Mato Grosso were found to be high yielders compared to those from Acre and Rondonia. The Mato Grosso genotypes also have a greater morphological resemblance to Wickham clones than those from Rondonia and Acre.

Table 5. Range and general mean of growth characters in the juvenile phase in 80 accessions

Character	Range				Population mean	Control RRII 105	Computed F	CD value
	Minimum	Genotype	Maximum	Genotype				
Girth (cm)	4.72	MT 929	10.41	RO 322	7.38	6.06	7.02**	1.34
Height (cm)	100.89	MT 929	322.99	RO 395	191.98	145.70	5.26**	55.0
No of leaf whorls	4.89	MT 906	9.12	RO 894	6.73	6.78	6.46**	1.14
No of lvs	41.20	MT 929	119.80	RO 322	69.80	62.80	6.44**	19.89
LAI	0.08	MT 929	0.46	RO 322	0.19	0.13	4.84**	0.11

** Significant at $P < 0.01$ LAI: Leaf area index

Table 6. Range and mean of girth, yield and bark characters in the third year

Character	Range				Mean	Control RRII 105	Computed F	CD value
	Minimum	Genotype	Maximum	Genotype				
BT (mm)	2.00	RO 886	4.00	RO 395	2.86	3.06	164.3**	0.1
Soft BT in %	31.26	RO 287	64.88	RO 868	42.17	38.01	49.31**	3.1
Hard BT in %	35.12	RO 868	68.74	RO 287	57.83	61.99	76.0**	3.1
LVR	2.99	MT1031	11.01	RO 399	5.81	8.00	81.93**	0.53
DLV/row/ mm	11.50	RO 399	25.00	RO 894	17.15	23.76	146.34**	0.7
Diameter (μm)	13.44	MT 906	34.00	MT 899	21.46	16.30	58.63**	1.4
Girth (cm)	10.38	MT 929	27.06	RO 322	18.64	17.84	193.0**	4.7
Yield ($gt^{-1}t^{-1}$)	0.0481	RO 257	4.2711	MT1057	0.5273	2.3547	8.99**	0.5

BT- Bark thickness; TLVR- Total number of latex vessel rows; DLV- Density of latex vessels.
** - Significant at $P < 0.01$

Moderate estimates of phenotypic and genotypic coefficients of variation (P.C.V and G.C.V respectively) with high estimates of broad sense heritability were recorded for girth, height, number of leaf flushes, total number of leaves, leaf area index, bark thickness, soft and hard bark thickness, number latex vessel rows, density and diameter of latex vessels where as it was high for test tap yield (Table 7). This indicates the availability of high genetic variability and the highly heritable nature of these characters.

Table 7. Coefficients of variation and broad sense heritability for growth and bark structural characters in a set of 80 wild genotypes.

Character	PCV(%)	GCV (%)	H^2(%)
Girth of the plants (cm)	29.19	23.1	62.67
Height of the plants (cm)	26.28	19.68	56.08
Number of leaf flushes per plant	18.13	13.78	57.71
Total number of leaves per plant	30.37	23.06	57.65
Leaf area index	50.48	35.34	48.99
Total bark thickness (mm)	15.73	15.53	97.61
Soft bark thickness (%)	16.60	15.87	90.50
Hard bark thickness (%)	12.16	11.57	91.00
Total no of latex vessel rows	30.75	30.01	95.24
Density of latex vessels per row per mm	18.33	18.08	97.32
Diameter of latex vessels (um)	18.05	17.42	93.20
Test tap yield ($g\ t^{-1}\ t^{-1}$)	117.01	95.54	66.67

Genetic divergence studies in wild germplasm assume great importance in the context of their utilization in hybridization programs. A study on genetic divergence in a group of 80 wild genotypes of all the three provenances using the Mahalanobis D^2 Statistic for 14 selected morphological, leaf and bark structural characters in the immature phase grouped them into nine genetically divergent clusters (Table 8). The intra and inter cluster distances were worked out and the maximum inter cluster distance indicating the widest genetic diversity between clusters was estimated between clusters 3 and 4 (Abraham, 2001).

Table 8 Distribution of 80 wild genotypes in divergent clusters

Cluster no	No of genotypes	Genotypes included
1	20	MT 948, MT 901, RO 381, AC 604, AC 627, MT 1064, RO 257, AC 694, MT 1007, AC 626, RO 868, AC 966, AC 979, RO 380, RO 364, RO 330, RO 328, AC 657, AC 706, MT 1063.
2	17	AC 654, RO 883, AC 953, RO 317, RO 316, MT 1031, RO 352, RO 886, RO 256, RO 338, RO 319, MT 929, AC 986, RO 876, RO 859, MT 1021, MT 920.
3	8	RO 311, AC 963, MT 1055, RO 894, AC 644, AC 733, AC 754, AC 650.
4	4	AC 647, AC 995, MT 899, MT 947.
5	8	MT 928, MT 1011, RO 255, AC 1043, MT 931, MT 906, RO 369, RO 322.
6	7	RO 287, MT 1077, RO 879, AC 1090, MT 1028, MT 944, MT 935.
7	6	MT 1024, MT 945, AC 632, RO 395, MT 1057, RO 399.
8	5	MT 1005, MT 1029, AC 959, AC 453, RO 254.
9	6	MT 1008, RRII 105, MT 1030, AC 629, AC 426, MT 1025.

5.2. Further evaluation and selection

A set of 80 selections (Acre-22, Rondonia-20 and Mato Grosso-38) based on their superior performance for various desirable characters in the PETs has been subjected to evaluation in an FET in simple lattice design (4 replications, plot size 4) for yield and yield related secondary traits including timber potentialities besides in-depth studies on specific areas of interest. A list of 18 accessions with superior and/or comparable performance with that of the control at the age of four years is furnished in table 9.

Table 9. Genotypes showing better performance over control

Sl.No.	Genotypes	Girth	BT	LVR	DLV
1	MT 197	S (20.11)	S (3.39)	P (3.00)	P (33.52)
2	MT 999	P (15.49)	P (3.05)	S (5.48)	P (33.50)
3	MT1707	P (18.26)	P (2.98)	P (3.01)	P (31.49)
4	MT 915	S (19.40)	P (2.98)	P (3.01)	P (30.22)
5	MT 54	P (18.65)	S (3.34)	P (3.25)	NS (27.57)
6	MT1674	S (19.48)	P (2.90)	P (2.75)	NS (26.25)
7	RO 255	S (19.93)	P (2.96)	P (4.45)	NS (26.42)
8	MT 182	P (17.37)	S (3.22)	P (4.00)	P (31.18)
9	MT 919	S (19.13)	P (2.64)	P (4.25)	NS (28.28)
10	MT2227	P (15.52)	S (3.37)	P (3.51)	NS (29.45)
11	AC2004	P 19.07)	S (3.11)	P (2.99)	NS (24.78)
12	MT1630	S (19.57)	P (2.86)	P (2.75)	P (30.51)
13	MT 941	P (19.45)	S (3.21)	NS (2.24)	NS (27.42)
14	RO1517	P (18.57)	P (2.65)	S (5.51)	NS (23.77)
15	MT 68	P (17.36)	S (3.38)	P (2.73)	NS (29.93)
16	MT2464	P (14.46)	S (3.27)	NS (2.24)	P (32.15)
17	AC 692	S (20.19)	P (2.86)	NS (2.03)	NS (27.78)
18	AC2584	P (17.25)	S (3.14)	NS (1.99)	NS (24.01)
Control	RRII 105	15.58	2.52	4.01	33.29

BT- Bark thickness: TLVR - Total no of latex vessel rows: DLV- Density of latex vessels.
S -Significantly higher than control; Figures in parentheses are mean values
P - On par with control NS -Non significant

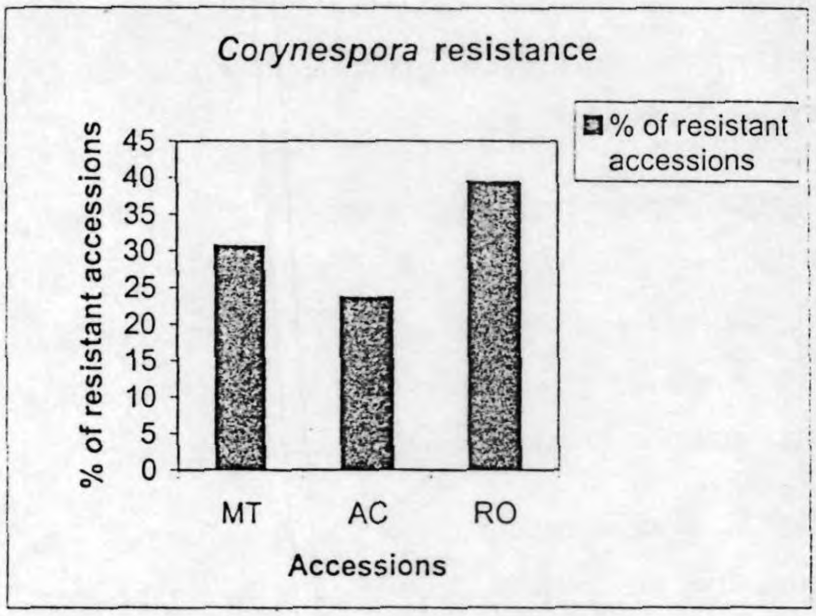
Data on growth performance at the age of 4 years for the traits girth bark thickness, number and density of laticifers and test tap yield indicated significant differences for all the characters studied. Majority of the genotypes were statistically on par with the control RRII 105 in growth while superiority was observed in seven accessions for girth; nine for bark thickness and two for number of latex vessel rows. During the 6th year of growth, 64 genotypes, (17 Acre, 17 Rondonia and 30 Mato Grosso) recorded a summer girth on par with RRII 105, whereas the drop in summer girth was negligible. The annual girth of 73 genotypes (Acre- 19; Rondonia- 19 and Mato Grosso- 35) was on par with RRII 105 at the age of 7 years. One MT genotype, recorded a yield statistically on par with RRII 105 while four Mato Grosso, two Rondonian and oneAcre genotype recorded 20 – 35% of the yield of RRII 105. Certain genotypes showed early indication of timber potentialities as revealed by their high growth rate, bole habits and branching habits. The genotypes were ranked based on the pooled performance of all the five characters recorded. Among the top 25% of the wild accessions, 20 % were of Mato Grosso provenance.

6. Screening for resistance to biotic stress (major diseases)

In India, the two major leaf diseases viz. abnormal leaf fall disease (*Phytophthora* sp.) and powdery mildew (*Oidium* sp.) cause considerable crop loss. Recent reports on the incidence of *Coryenespora* leaf spot observed in the popular clones RRII 105 and RRIM 600, in the rubber growing tracts of Karnataka and certain parts of North Kerala (Jacob, 1996), has caused serious concern over the possibility of the disease attaining epidemic proportions. In the case of the emergence of a virulent strain of a particular pathogen in favorable environmental conditions, the disease will spread and cause serious damage, if sufficient genetic variability is not available in the population. Wild germplasm being a rich source of genes conferring resistance to stresses, screening of the accessions for major diseases is a priority area.

A collaborative programme with the Pathology Division of the Institute is in progress for field and laboratory screening of the wild genotypes in a phased manner, for resistance to *Phytophthora, Colletotrichum and Corynespora* leaf diseases. Field screening of 3561 wild genotypes in six conservation nurseries for *Oidium* resistance over a period of two years revealed that 140 accessions had relatively higher resistance (< 25 % disease incidence). Laboratory screening of the genotypes for resistance to *Phytophthora* and *Corynesopra* has been initiated. Initial screening for resistance to *Phytophthora* in 61 genotypes showed seven accessions with good resistance. Similarly, out of 770 accessions screened 70 accessions showed high-level resistance to *Corynespora* (Fig 1). Further systematic screening is envisaged to confirm the degree of disease reaction to different diseases.

Fig. 1. Provenance wise resistance to *Corynespora*



7. Screening for resistance to abiotic stress (drought and cold)

Studies have been initiated for screening of wild germplasm for drought and cold tolerance with the ultimate objective of developing location specific clones for non-traditional rubber growing areas. Field level screening of the wild accessions in the drought and cold prone areas in Dapchari (Maharashtra) and in the sub Himalayan region at Nagrakatta (Northern part of W.Bengal) respectively has been initiated.

7.1. Screening for drought resistance

Experiments on genotypic evaluation for assessment of the genetic variability and screening of wild *Hevea* germplasm for drought tolerance have been taken up. A preliminary screening was carried out in 100 wild genotypes based on cellular membrane stability, using a combination of water and heat stresses. Based on this study, 10 selected genotypes were subjected to in depth studies on various drought- related morphological, anatomical, physiological and biochemical characters along with control clones viz.RRII 105, RRIM 600 and Tjir 1 with the objective of identifying a set of reliable parameters for selection of drought tolerant genotypes. The parameters studied include plant height. number of flushes, total number of leaves, single leaflet area, girth, girth increment, inter-flush distance and specific leaf weight (morphological), number of stomata per unit area, leaf thickness, midrib diameter, thickness of palisade and mesophyll layers, no of palisade cells per unit length, total number of latex vessel rows

(LVR), no. of LVR in soft bark, total bark thickness and thickness of soft bark (anatomical) and transpiration rate, stomatal resistance, leaf temperature, soil and leaf water potential, chlorophyll fluorescence, total chlorophyll content, chlorophyll reduction percentage and epicuticular wax content (physiological and biochemical) besides dry matter stress tolerance index in a glass house experiment.

The data revealed that parameters such as girth increment and rate of stomatal conductance during stress period, dry matter stress tolerance index, chlorophyll fluorescence, chlorophyll and epicuticular wax contents, palisade layer thickness and number of palisade cells per unit length are among the reliable indices for screening genotypes for drought tolerance, which need to be confirmed in further experiments.

The genotypes were ranked based on parametric relationships using the rank sum method (Table 10). The rank sums varied from 65 to 121 with MT 41 scoring the highest rank sum followed by MT 55 and AC 650, indicating their superior performance.

Table 10. Ranking of selected genotypes based on parametric relationships

GT *	PH *	BD *	NL *	LS *	GID *	DMSI *	SC *	TR *	Fv/ Fm *	ECW *	CC *	NS *	PTT *	SBP *	SBL *	CR *	Rank sum
AC 1044	3	2	4	6	1	2	9	10	10	3	8	2	8	2	9	8	87
MT 55	10	7	8	9	4	3	8	8	9	7	7	4	4	8	5	1	102
AC 446	5	8	3	8	3	5	7	6	7	5	1	10	3	1	6	10	88
MT 41	9	5	10	4	10	8	10	5	5	10	10	7	2	9	10	7	121
MT 76	8	4	7	1	7	4	4	4	3	8	6	5	9	6	3	3	
MT 66	2	9	2	3	6	6	3	3	1	2	9	1	5	4	4	5	65
MT 938	7	3	6	7	8	1	1	2	4	1	3	9	1	10	1	9	72
AC 650	6	10	9	5	5	9	5	7	6	4	2	8	6	5	8	2	97
AC 652	1	1	1	2	9	10	6	9	8	9	4	6	10	7	7	6	96
AC 728	4	6	5	10	2	7	2	1	2	6	5	3	7	3	2	4	69

* GT- Genotypes; PH - Plant height; BD - Basal diameter; NL- No. of leaves ; LS – Leaf size; SLA Single leaflet area; GID - Girth increment/decrease; DMSI-Dry Matter Stress Tolerance Index SC - Stomatal conductance ; TR - Transpiration rate; Fv/Fm- Chlorophyll fluorescence ECW- Epicuticular wax ; CC - Chlorophyll content; NS - No. of stomata; PTT - Palisade tissue thickness ; SBP - Soft bark proportion % ;SBL- Soft bark LVR % CR- Chlorophyll reduction %.

7.1. Field screening for drought tolerance

Screening wild genotypes in the drought prone area at, Dapchari in Maharahtra state was initiated. Wide variability was noticed in sprouting percentage of budded stumps recorded during the drought season of 2001.Out of 156 accessions 33 showed no sprouting at all while 11 accessions recorded above 90% sprouting. All the three control clones responded in accordance with their known drought response. RRII 105, with medium tolerance had a sprouting percentage of 51.28, Tjir 1 known for its drought susceptibility had only 33.23% sprouting and RRIM 600, a known drought tolerant clone had a sprouting percentage of 70.8%.

A set of 63 genotypes from the above experiment was planted for field evaluation. Growth performance in the post drought period in the juvenile phase indicated wide variability (Table 11). Among the accessions, MT 54 was found to have the maximum value for the growth characters height, girth and no of leaf whorls after the stress period while RO 3461 was the least vigorous.

Table 11: Range in post drought growth performance in a set of 63 genotypes.

Character	Minimum (genotype)	Maximum (genotype)	Population mean	Control clones		
				RRII 105	RRIM 600	Tjir I
Height (cm)	76.7 (RO 3461)	368.00 (MT 54)	199.84	44.26	71.60	34.37
Girth (cm)	9.27 (RO 3461)	30.55 (MT 54)	19.03	18.53	22.69	10.78
No of Whorls	2.8 (RO 2504)	5.5 (MT 54)	3.95	3.7	4.73	3.68
No of Leaves	26.0 (RO 3461)	83.4 (MT 1627)	49.49	44.26	86.84	34.37
RWC*	0.7692 (MT1589)	0.9830 (MT 1595)	0.900	0.8862	0.8850	0.9029

*Relative water content

The top 10 accession each, which showed superiority for the growth characters and relative water content in the above experiment is listed in Table 12. It is evident that Mato Grosso genotypes were in general superior compared to the accessions from the other two provenances. Among the accessions ten recorded very high percentage girth increment (>100 %), over the drought period indicating their potential to withstand drought.

Table 12:List of superior genotypes based on their post drought growth performance.

Height	Girth	Number of whorls	Number of leaves	Relative Water Content
MT 54	**MT 54**	**MT 54**	**MT 1627**	MT 1595
MT 1649	**MT 1616**	MT 1589	**AC 1609**	**MT 54**
MT 1627	**MT 1649**	**MT 1649**	MT 1668	AC 2532
MT 1616	**AC 3307**	**RO 268**	**MT 54**	RO 3461
MT 67	**MT 1627**	**AC 3307**	**MT 1616**	MT 899
AC 1609	MT 1619	MT 1579	**MT 1668**	**RO 268**
MT 1668	**AC 1609**	**MT 67**	MT 1579	**MT 67**
AC 4160	**AC 4160**	**AC 4160**	AC 157	**MT 1649**
RO 268	**MT 67**	**MT 1616**	MT 2594	AC 2692
MT 1619	MT 1591	**MT 1668**	**RO 268**	**MT 1616**

Accessions superior for more than one character are given in bold print

7.2. Field screening for cold resistance

Experiments on screening the wild genotypes for cold tolerance have been laid out in a cold prone area at the Regional Station, Nagrakata, at the Northern part of the State of West Bengal. A total of 64 wild genotypes were planted in two trials along with the wickham clones SCATC 93/114, RRIM 600 and RRII 105, Haiken 1, RRII 203 and PB 235. Genotypes were selected based on their survival and growth during the previous cold season at Nagrakatta. A set of growth characters – girth, height, number of leaf whorls and total number of leaves were recorded during the pre and post winter period in the first year of planting. Statistical analysis of this data reveals significant differences among the genotypes for all the characters studied. Genotype MT 1058 showed 100 percent cold susceptibility where all the plants were lost. But another Mato Grosso genotype MT 900 along with two Rondonian genotypes, RO 2902 and RO 2565 was found to be very vigorous in terms of its girth and height after passing through the stress period. The range and mean values of the wild genotypes in the post winter period in comparison to selected control clones is given in Table 13. The clones were ranked according to their increment between the pre and post winter absolute mean values for the growth characters and the top ranking 10 genotypes are listed in Table 14.

Table 13. Post winter data on range and mean of growth characters in juvenile phase

Characters	Min.	Genotype	Max.	Genotype	G Mean	Controls	
						PB 235	RRII 105
Girth (cm)	3.243	AC 3293	22.99	RO 2902	18.72	16.16	20.19
Height (cm)	87.48	AC 3074	219.1	RO 2565	167.19	154.97	177.71
No of whorls	3.47	AC 3074	6.58	RO 2277	4.98	4.82	5.17
No of leaves	3.0	AC 3075	476	MT 2229	25.50	31.21	29.58

Table 14. Superior genotypes identified based on growth rate during winter season

Girth	Height	No.of whorls	No.of leaves
MT 900	MT 1020	AC 3293	**RO 3229**
RO 2809	**RO 2809**	MT 1020	**MT 1020**
AC 3300	**RO 2565**	**RO 2277**	**RO 3043**
RO 2902	**RO 3043**	AC 490	MT 5131
RO 2908	**RO 2387**	**RO 3043**	AC 3057
RO 2889	**RO 2277**	**RO 2565**	**RO 2901**
AC 3514	AC 3293	**RO 2948**	**RO 2889**
RO 322	**RO 2886**	AC 3810	AC 3074
RO 2387	**RO 2901**	**RO 4542**	**RO 2948**
RO 2277	**RO 3197**	MT 2229	**RO 4542**

Accessions superior for more than one character are given in bold print

Maximum increment in growth characters during the stress period was recorded in MT 900 and MT 1020 for girth and height of the plants respectively. AC 3293 had the highest increment for number of leaf whorls and RO 3229 had a positive increment in the number of leaves produced in the stress period. MT 1020 also ranked high for the number of leaf whorls and number of leaves produced during the stress period.

8. Screening for timber characteristics

The contribution of rubber wood products to rubber industry is in the increase with the increasing demand of timber for wood based industries. It is imperative that rubber growers have a choice of clones with a high timber yield in addition to latex yield, which necessitates development of timber-latex clones. Rubber trees with desirably large stem diameter and longer boles (branching at higher elevations from the ground) with less branching would give high timber yield. Considering the inherent demerits of rubber wood preventing specific end uses, the need for extensive and coordinated investigations to improve the quality and durability of rubber timber assumes much significance.

Screening of *Hevea* germplasm for both quantitative and qualitative timber characteristics with the above objectives has been initiated.

8.1. Screening for timber quantity

Screening of genotypes and the identification of accessions with desirable timber quantity traits has been initiated. A set of 19 wild genotypes selected based on vigorous growth, bole and branching habits and juvenile yield along with six Wickham clones were planted in the field employing a simple lattice design with three replications at the Regional Research Station, N. Kerala in 2000.

The genotypes selected for field evaluation are AC 635, MT 935, AC 637, MT 922, AC 650, MT 919, AC 651, MT 915, AC 707, MT 941, MT 999, MT 1032, AC 685, AC 655, RO 322, RO 879, RO 255, MT 1020, MT1021, RRII 105, RRII 33, RRII 118, RRIM 600, PB 235 and PB 260.

Data on juvenile girth revealed that the wild genotypes recorded comparable /higher girth with that of Wickham clones. Out of 19 wild genotypes, 14 and four each measured a girth significantly higher than PB 260 and PB 235 respectively. Among the three provenances, Acre genotypes showed vigorous growth with long straight boles, sparse and high leveln branching habit in contrast to the short and stout bole with profuse branching habits of Mato Grosso genotypes.

8.2. Screening for timber quality traits

Anticipating a scenario of wide spread cultivation of rubber for quality timber production in addition to latex yield, it is highly essential to develop clones combining high timber production potential with desirable quality and durability. Being a potential plantation hard wood for versatile utilization, the improvement of quality and durability of rubber wood has been identified as a priority area. The major limitations of rubber wood for its versatile utilization are (i) natural defects and (ii) biological defects. The natural defect is caused by high incidence of tension wood and low level of lignification leading to considerable reduction in physical/ strength properties, uncontrollable distortions and dimensional instability of finished products as well as high incidence of wind damage. Biological defects are due to the high proportion of reserve metabolites in the form of soluble sugar and starch leading to biological deterioration.

Increasing the level of lignification in rubber wood will help to minimize the above demerits to a considerable extent. In this context a research programme has been initiated with the objective of improving the quality and durability of rubber wood through lignin biosynthesis studies. Standardization of protocols for identification, localization, and quantification of lignin precursor enzyme markers is in progress.

9. Hybridization programmes

Superior wild accessions have been incorporated as one of the parents in Wickham x Amazonian hybridization programs in various rubber research institutes. In India, among the first of such crossings done in 1989, incorporating seven wild genotypes, hybrid seedling progeny of the cross combination RRII 105 x RO 87 recorded superior test tap yield in the early growth phase (RRII, 1994). Out of 43 clones evolved from this programme planted in two small-scale trials in 1995, 21 showed higher girth than the popular clone RRII 105 in the 6[th] year after planting, with standard heterosis ranging from 2.62 to 74.29%.

Another set of nine superior genotypes was used in crosses in 1997 with two popular Wickham clones RRII 105 and RRIM 600 and the resultant 400 hybrid seedling progenies from 14 cross combinations are under evaluation. The average girth and height of the seedlings in the 3[rd] year are given in Table 15. Test tapping these seedlings in the nursery at the age of 27 months revealed 12 promising genotypes with cumulative yields ranging from 3.03 to 12.76 g from 10 tappings. In the Wickham X Amazonian hybridization programme of 2002, some of the latest high yielding Wickham clones evolved at RRII were also included as female parents. However, there is a long way to go for realizing the expected benefits from such a large collection of wild germplasm.

Table 15. Performance of hybrid seedlings in the third year of growth in terms of girth and height.

Cross combinations	Progeny size (n)	Mean girth (cm)	CV (%)	Mean height (cm)	CV (%)
1. RRII 105 X MT 1027	6	14.06	32.82	321	38.62
2. RRII 105 X MT 1005	54	12.81	21.38	381	18.32
3. RRII 105 X MT 999	13	12.81	25.52	430	32.84
4. RRII 105 X MT 1014	12	11.50	19.64	575	24.21
5. RRII 105 X MT 1021	5	15.00	19.45	622	11.33
6. RRIM 600 X MT 1027	15	15.46	13.42	585	15.78
7. RRIM 600 X MT 1005	45	12.81	23.68	563	22.25
8. RRIM 600 X MT 999	50	13.99	26.63	572	26.50
9. RRIM 600 X MT 1014	21	13.81	14.62	581	18.65
10. RRIM 600 X MT 1021	44	14.56	23.32	563	21.84
11. RRIM 600 X AC 495	54	15.23	22.24	545	20.63
12. RRIM 600 X AC 498	31	10.11	25.23	533	26.86
13. RRIM 600 X AC 817	23	14.56	15.75	484	13.44
14. RRIM 600 X RO 380	27	13.73	24.29	553	19.85

10. Future programmes

In the context of diminishing per capita available land and water resources, expanding biotic and abiotic stresses compounded by the threat of the climate changes, conservation and utilization of natural resources assumes much significance internationally. Morphological characterization, which is done for the juvenile phase will have to be completed for the mature phase too to create a complete database on the wild accessions for their effective utilization. Computerization of the voluminous database on *Hevea* germplasm is now possible, with the creation of user-friendly software for easy storage and retrieval of data.

Molecular techniques like RFLP, RAPD and microsatellites provide reliable genetic information for molecular characterisation, evaluation and utilisation of germplasm resources. Data on genetic distance is required for identification of diverse sub populations. Application of molecular markers can be valuable adjuncts to conventional breeding for better exploitation of the available genetic variability in the Wickham gene pool and the wild germplasm to bring about significant contributions for further genetic improvement.

Establishment of a core population is essential so that greater attention can be given to these accessions. This can be based on data on genetic distance between the accessions, in the absence of which, random stratified sampling of accessions based on their provenance may be adopted. Molecular tools may be used to supplement data on genetic distances obtained using morphological characters.

The challenge before the breeder lies in evolving strategies for identification of the desired variability from the available genetic resources Wild germplasm is definitely a source of abundant variability required for development of location specific clones capable of withstanding different constraints prevalent in non traditional areas, like extremes of temperature in winter and summer, prolonged drought, high velocity winds, high altitudes, depleted soils etc.

Systematic screening programmes are essential for locating the sources of superior genes. In addition, sustained efforts towards identification of molecular markers linked to specific traits of interest through Marker Assisted Selection (MAS) are required for achieving speed and reliability in the selection process.

Incorporation of the desired genes in to the breeding pool through hybridization programmes has to be intensified. In *Hevea,* hybridization between widely divergent genotypes is expected to result in greater range of variability especially in view of the highly heterozygous nature of the crop. Establishment of a garden comprising selected wild accessions and wickham clones is expected to result in superior half sib progeny for selection of desired recombinants.

There is no doubt that the wild germplasm is the best raw material not only to achieve the current thrust areas like development of location specific clones capable of withstanding various diseases, cold, drought, high elevation etc. but also to meet the future demands of the industry.

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ANNEXURE 1: List of accessions in the text, along with the IRRDB codes

Accession	IRRDB code	Accession	IRRDB code	Accession	IRRDB code
AC 157	AC/T/1-5/36	MT 66	MT/IT/14-30/93	MT1668	MT/IT/15-28/131
AC 426	AC/F/5-21/117	MT 67	MT/IT/14-30/95	MT1674	MT/IT/15-28/142
AC 446	AC/F/5-21/208	MT 68	MT/IT/14-30/100	MT1707	MT/IT/15-28/207
AC 453	AC/F/5-21/220	MT 76	MT/IT/14-30/131	MT2227	MT/C/2-10/169
AC 490	AC/S/8-35/66	MT 1055	MT/C/10-17/20	MT2229	MT/C/1-18/30
AC 495	AC/S/8-35/78	MT 182	MT/C/2-10/8	MT2464	MT/IT/12-26/75
AC 498	AC/S/8-35/88	MT 197	MT/C/2-10/39	MT2594	MT/IT/16-34/159
AC 604	AC/S/11-41/1	MT 899	MT/IT/16-34/2	MT5131	MT/IT/13-29/11
AC 626	AC/S/11-41/111	MT 900	MT/IT/16-34/5	RO 254	RO/OP/4-20/12
AC 627	AC/S/11-41/113	MT 901	MT/IT/16-34/6	RO 255	RO/OP/4-20/15
AC 629	AC/S/11-41/115	MT 906	MT/IT/16-34/12	RO 256	RO/OP/4-20/27
AC 632	AC/S/11-41/198	MT 915	MT/IT/16-34/56	RO 257	RO/OP/4-20/49
AC 635	AC/S/11-41/240	MT 919	MT/IT/16-34/79	RO 268	RO/OP/4-20/97
AC 637	AC/S/11-41/253	MT 920	MT/IT/16-34/81	RO 287	RO/OP/4-20/139
AC 644	AC/S/11-41/282	MT 922	MT/IT/16-34/94	RO 311	RO/C/9-23/127
AC 647	AC/S/11-41/327	MT 928	MT/IT/16-34/133	RO 316	RO/C/9-23/141
AC 650	AC/S/11-41/348	MT 929	MT/IT/16-34/135	RO 317	RO/C/9-23/142
AC 651	AC/S/11-41/356	MT 931	MT/IT/16-34/138	RO 319	RO/C/9-23/152
AC 652	AC/S/11-41/364	MT 935	MT/IT/16-34/174	RO 322	RO/C/9-23/157
AC 654	AC/S/9-39/5	MT 938	MT/IT/16-34/184	RO 328	RO/C/9-23/174
AC 657	AC/S/9-39/15	MT 941	MT/IT/16-34/199	RO 330	RO/C/9-23/179
AC 685	AC/S/9-39/105	MT 944	MT/IT/16-34/208	RO 338	RO/C/9-23/203
AC 692	AC/S/9-39/128	MT 945	MT/IT/16-34/210	RO 352	RO/C/9-23/308
AC 694	AC/S/9-39/136	MT 947	MT/IT/16-34/212	RO 364	RO/C/8-24/98
AC 706	AC/S/12-42/18	MT 948	MT/IT/16-34/213	RO 369	RO/C/8-24/130
AC 707	AC/S/12-42/23	MT 999	MT/C/1-18/9	RO 380	RO/C/8-24/177
AC 728	AC/S/10-37/7	MT1005	MT/C/1-18/60	RO 381	RO/C/8-24/179
AC 733	AC/S/10-37/21	MT1007	MT/C/1-18/68	RO 395	RO/C/8-24/302
AC 754	AC/S/10-37/79	MT1008	MT/C/1-18/69	RO 399	RO/C/8-24/327
AC 817	AC/F/7-38/85	MT1011	MT/C/1-18/75	RO 859	RO/CM/12-62/26
AC 953	AC/F/6B-40/11	MT1014	MT/C/1-18/78	RO 868	RO/CM/12-62/44
AC 959	AC/F/6B-40/21	MT1020	MT/C/1-18/90	RO 876	RO/CM/12-62/91
AC 963	AC/F/6B-40/27			RO 879	RO/CM/12-62/100
AC 966	AC/F/6B-40/35	MT1021	MT/C/1-18/91	RO 883	RO/CM/12-62/110
AC 979	AC/F/6B-40/79	MT1024	MT/C/1-18/99	RO 886	RO/CM/12-62/115
AC 986	AC/F/6B-40/104	MT1027	MT/C/1-18/107	RO 894	RO/CM/12-62/136
AC 995	AC/F/6B 40/169	MT1028	MT/C/1-18/108	RO 894	RO/CM/12-62/136
AC1043	AC/S/12- 42/365	MT1029	MT/C/1-18/19	RO1517	RO/A/7-25/329
AC1044	AC/S/12-42/366	MT1030	MT/C/1-18/113	RO2277	RO/J/5-33/38
AC1090	AC/F/6A-36/121	MT1031	MT/C/1-18/116	RO2387	RO/C/9-23/45
AC2004	AC/S/10-37/336	MT1032	MT/C/1-18/117	RO2504	RO/A/7-25/423
AC2532	AC/F/7-38/16	MT1057	MT/C/10-17/24	RO2565	RO/J/6-32/26
AC2584	AC/B/19-56/239	MT1063	MT/C/10-17/94	RO2809	RO/CM/10-44/391
AC2692	AC/S/10-37/349	MT1064	MT/C/10-17/102	RO2886	RO/CM/10-44/670
AC3057	AC/S/8-35/394	MT1077	MT/C/7-8/37	RO2889	RO/CM/10-44/683
AC3074	AC/S/8-35/862	MT1579	MT/IT/12-26/3	RO2901	RO/CM/10-44/735
AC3293	AC/AB/15-54/337	MT1589	MT/IT/12-26/77	RO2902	RO/CM/10-44/738
AC3300	AC/AB/15-54/418	MT1591	MT/IT/12-26/86	RO2908	RO/CM/10-44/768
AC3307	AC/AB/15-54/442	MT1595	MT/IT/12-26/128	RO2948	RO/PB/1-2/104
AC3514	AC/F/6A-36/426	MT1609	MT/IT/17-27/69	RO3043	RO/A/7-25/562
AC3810	RO/A/7-25/379	MT1616	MT/IT/17-27/93	RO3197	RO/CM/11-63/387
AC4160	AC/S/12-42/60	MT1619	MT/IT/17-27/101	RO3229	RO/CM/11-63/454
MT 41	MT/IT/14-30/12	MT1627	MT/IT/15-28/62	RO3461	RO/CM/12-62/79
MT 54	MT/IT/14-30/38	MT1630	MT/IT/15-28/70	RO4542	RO/PB/1-2/4
MT 55	MT/IT/14-30/39	MT1649	MT/IT/15-28/99		