Advances in Tree Seed Science and Silviculture

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Front cover : Germination studies in Calophyllum inophyllum
(Photo: R. Anandalakshmi)

Back cover : Windbreak agroforestry system with superior clones of IFGTB to protect Banana crop (Age of trees-4 years)
(Photo: C. Buvaneswaran)

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CONSERVATION OF 1981 IRRDB *HEVEA* GERMPLASM COLLECTION IN INDIA - CURRENT STATUS

M.A. Mercy, G. Prabhakara Rao and C.P. Reghu

Abstract

The large share of 4548 wild accessions belonging to the 1981 IRRDB wild *Hevea* germplasm collection introduced into India between 1984 and 1990 are being conserved *ex situ* in both traditional and non-traditional regions. Systematic efforts are underway for characterization, evaluation, documentation and utilization of these valuable genetic resources in a phased manner. 3988 accessions have been characterized in the juvenile stage using 22 morphological traits. Wide diversity was observed in this collection. The accessions are now in various stages of field evaluation. A total of 132 potential accessions with relatively high yield have been identified for further evaluation and selection. Accessions showing potential for various secondary traits, tolerant to abiotic stresses (drought and cold), biotic stresses such as tolerant to *Oidium*, *Phytophthora* and *Corynespora* diseases have also been identified for incorporation in the breeding programmes. W x A hybridization programmes carried out since 1990 have resulted in some heterotic hybrids that have surpassed the popular high yielding clones in rubber and timber. There are indications of direct selection as well, for yield, from the wild germplasm which are being evaluated in the On-farm trials as part of the participatory clone evaluation programme.

The present paper is a comprehensive report on the progress and major activities on conservation, charactersization, evaluation, screening for timber quality traits, abiotic and biotic stress resistance and utilization of the selections as parents for future breeding programmes.

Key words : Abiotic stress resistance, Biotic stress resistance, Characterization, Conservation, Evaluation, Genetic variation, *Hevea brasiliensis*, Introduction, Utilization, Wild germplasm,

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Introduction of wild *Hevea* germplasm to India

To broaden the extremely narrow genetic base of cultivated rubber, and to collect and conserve the fast disappearing wild germplasm of *Hevea*, the IRRDB had conducted an expedition jointly with EMBRAPA of Brazil in 1981, in the Amazon forests of Brazil. The expedition was resulted in the collection of more than 64736 seeds and 194 ortets from 3 states in Brazil, covering a total of 64 locations in 16 districts (IRRDB, 1982). Half the seed collection was retained at Brazil as per the agreement with them, while the remaining half was distributed to the two base centers at Ivory Coast and Malaysia for further multiplication and redistribution to IRRDB member countries. Each seed was given an accession number, and the vegetative derivatives carried the same number. On the other hand, the entire collection of ortets was first multiplied at an intermediate station at Guadeloupe, before re-transmission to Malaysia. Highly stringent phytosanitary measures at each stage ensured that diseases, especially SALB, were not transmitted along with the bud wood. The germplasm, both of seedling and of ortet origin, were distributed to member countries in the form of bud wood. India's share of the wild germplasm was introduced into the country between 1984 and 1990 in batches. Once the accessions were established in conservation nurseries, the next priority was to characterize and evaluate the germplasm for effective utilization in future crop improvement programmes.

Conservation

The wild germplasm introduced to India is being conserved in source bush nurseries (SBN) at two locations. Currently Rubber Research Institute of India is conserving 4548 wild *Hevea* accessions in field gene banks, one in traditional region in Kerala and the other in the non-traditional rubber growing regions of the North East, India. The provenance-wise status of wild accessions being conserved *ex situ* in India is depicted in (Table 1).

Characterization and evaluation

a. Conservation nurseries (SBNs)

A total of 3727 accessions were re-established in new conservation nurseries (SBNs) from 2003 onwards and preliminary evaluation and juvenile characterization were carried out in these new nurseries. Morphological characterization on 22 qualitative traits using descriptors (Rao *et al.*, 2005a) in

Table 1. Details of conservation of the 1981 IRRDB wild germplasm in India

Provenance	Districts 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

the first year of growth was done in all these nurseries. The Shannon-Weaver Diversity indices (SDI) estimated for the 22 morphological qualitative traits during characterization of 195 accessions of SBN 2003 showed high levels of diversity for most traits ranging from 0.0454 (leaf scar and leaf texture) to 0.999 (leaf margin) (Suma *et al.*, 2006).

Clustering of a set of 60 wild accessions using 22 RAPD primers showed genetic diversity in this collection (RRII, 2002). Another study involving RAPD profiling of 110 wild accessions using 16 informative primers indicated considerable genetic variation among the accessions from Acre, Rondonia and Mato Grosso provenances (Varghese *et al.*, 2002). The accessions were grouped into eight distinct clusters, which showed geographical distinctiveness to the three states from where these materials were originally collected. A few accessions which were distinctly divergent in the clusters were also identified. Biochemical characterization revealed that two wild accessions *viz.* AC 166 and AC 2004 had high ATP content in latex similar to that of the check clone RRII 105.

Preliminary evaluation of the accessions in the conservation nurseries helped to identify potential accessions for juvenile yield and other secondary characters. The evaluation carried out in a set of 55 accessions in SBN 2001 showed significant variability for test tap yield, growth and structural traits (Rao, 2009). Highest test tap yield was recorded in RO 5018 followed by RO 2841 and RO 5432. RO 5432 ranked top for girth and girth increment. MT 4771 recorded highest bark thickness and RO 2841 had the highest number of latex vessels. Diameter of latex vessels ranged from 10.69 µm (AC 5487) to 21.66 µm (MT4762). MT 4690 (5.14 m) recorded highest crotch height followed by RO 5442 (5.05 m) and MT 4772 (4.36 m). Rao and Varghese (2011) reported wide variability for various yield and growth traits in a set of 545 wild accessions in SBN 2003. Genetic divergence studies in wild germplasm assume great importance in the context of

their utilization in hybridization programs. Genetic diversity was estimated in a set of 545 accessions of SBN 2003, using the k-means statistic. Seven quantitative traits *viz.*, plant height, diameter of the plant at 30 cm height, no. of leaf whorls, internodes length, no. of leaves, leaf size in terms of single leaf area (cm^2) and total leaf area (cm^2) were studied in the early growth phase. The wide overall variability of the wild germplasm was reflected in the distribution patterns by forming 15 clusters (Rao *et al.,* 2005b). Among the 544 accessions of SBN 2003, AC 4394, RO 3446, AC 4111, RO 2876, AC 159 and RO 2798 were the accessions which gave good juvenile yield.

Out of 975 wild accessions in conservation nursery SBN 2004, based on two rounds of test tapping, 13 accessions having 80% or more yield of RRII 105 were identified and selected for Further Evaluation Trial and 15 accessions having 50-80% yield of RRII 105 were identified and selected for Clonal Nursery Evaluation. Test tapping of 806 accessions in SBN 2006 identified 63 accessions with more than 50% test tap yield with that of RRII 105 for second round of test tapping. The SBN 2007 comprising of 500 accessions was monitored for early growth and test tap yield. Data were recorded on the traits yield ($gt^{-1}t^{-1}$) by test tapping in 3rd year, girth (cm) in the 2nd year to 5th year, girth increment (cm/yr) over 3 years, number of branches, crotch height (m), plant height (m) in the 2nd year and 5th year and height increment (m). 25 potential accessions for yield were identified and MT 4788, AC 226, AC 4155, MT 1007 were top yielders (Rao *et al.,* 2012). The accession RO 3792 recorded highest girth followed by AC 734 and MT 1680. Wide range of variability was observed for various yield and growth contributing traits (Table 2).

b. Preliminary evaluation trials (PETs)

Earlier, wild accessions were evaluated up to mature stage and selected ones were advanced to further evaluation. At RRS, Padiyoor, monthly yield and annual girth was recorded in 171 wild accessions in PET 2000A. Accession AC 2537 recorded the highest girth followed by RO 2171 and AC 3609. Highest annual yield was recorded in accession RO 2786, AC 3131 and AC 552. Monthly yield and annual girth was recorded in PET 2000B at RRS, Padiyoor. Among 166 wild accessions, AC 647 recorded the highest girth of 77.0 cm followed by accession RO 2883 (73.25 cm) whereas the check clones RRII 105, RRII 208 & RRIM 600 recorded girth of 53.21 cm, 46.14 cm and 43.98 cm respectively. 27 accessions had girth higher than RRII 105 showing their timber potential. For mature yield,

Table 2. Range of variability for yield and growth characters in wild *Hevea* germplasm

Characters	Wild accessions			Control clones				CV (%)
	Maximum	Maximum	General mean	RRII 105	RRII 208	RRIM 600	PB 235	
Test tap yield ($gt^{-1}t^{-1}$)	0.01 (MT5081)	3.52 (MT4788)	0.15	0.112	0.052	1.422	0.20	245.17
Girth (cm) - 2nd year	3.00 (AC493, RO 1374, RO2367)	15.90 (AC1218)	7.81	5.69	4.77	6.81	4.98	42.83
Girth (cm) - 3rd year	6.00 (MT4350)	21.00 (RO3792)	12.06	9.23	8.86	12.20	8.94	30.72
Girth (cm) - 4th year	8.00 (AC3944)	28.25 (MT2226)	16.59	12.92	12.34	17.15	12.20	31.29
Girth (cm) - 5th year	10.67 (RO2328)	37.50 (RO3792)	21.06	18.86	18.53	22.75	18.91	32.15
Girth increment (cm)	0.67 (RO319, AC3905)	10.00 (AC577, AC 734)	4.42	4.39	4.59	5.31	4.64	46.92
No of branches	1.67 (RO1754)	13.50 (MT1680, RO 4194)	4.86	3.81	4.14	4.56	3.97	53.76
Crotch height (m)	1.20 (RO4574)	9.60 (RO335)	3.38	2.42	2.58	3.03	2.85	26.00
Plant height (m) - 2nd yr	0.85 (MT136)	7.25 (MT2226)	2.83	1.84	1.54	2.61	1.91	55.25
Plant height (m) - 5th yr	1.50 (RO4574)	13.00 (RO229)	6.51	4.31	4.29	6.53	4.64	41.23
Height increment (m)	0.50 (RO4574)	9.80 (AC989)	3.66	2.46	2.75	3.83	2.79	44.71

Note: Figures in parenthesis denotes the name of accession.

among the 3 check clones, RRII 105 recorded the highest yield (44.22 g). Among the wild accessions, accession RO 341(43.01 g) recorded highest mature yield on par with RRII 105 followed by accession AC 2004 (31.73 g.). AC 567, AC 1964 and AC 824 recorded the highest girth in PET 2002.

As expected, most of 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. A total of 55 wild accessions were identified as potential high yielders from various preliminary studies so far conducted. All these accessions will be evaluated in detail in statistically laid out further evaluation trials to confirm their yield potential.

Further evaluation and selection

a. Clonal nursery evaluation

Accessions showing 50-80% test tap yield of RRII 105 are subjected to an evaluation in the clonal nursery at spacing of 2.5 x 2.5 m. for a period of five years and will be assessed for juvenile yield from third year onwards by test tapping. Along with yield assessment, they will be assessed for growth and vigour by recording girth. One such clonal nursery comprising 15 selected wild accessions from SBN 2004 is grown at Central Experiment Station, Rubber Research Institute of India, Chethackal, Ranni along with three check clones

b. Further evaluation trials

The first further evaluation trial comprising 80 selected accessions are under 18th year of evaluation at Regional Research Station, Padiyoor, Northern Kerala. Among these, five Mato Grosso accessions and one Acre accession showed the girth significantly higher than that of the control clone RRII 105 (Table 3). The accession AC 166 had the highest dry rubber yield (65.25 $gt^{-1}t^{-1}$) which is statistically on par with the yield of RRII 105 (64.04 $gt^{-1}t^{-1}$) in the 8th year of tapping. Three accessions (RO 2385, AC 2004 & RO 2908) had 85% to 95 % of the yield of RRII 105, while 11 accessions showed 50% to 80% of the yield of RRII 105 (Table 4). Five accessions showed better performance for timber yield compared to RRII 105 (Table 5). Two Mato Grosso accessions (MT 999 & MT

Table 3. Growth performance of wild accessions in FET 1995

Accession	Girth (cm)
MT 941	83.32
MT 1032	83.24
MT 1630	79.01
MT 915	77.39
MT 191	74.91
AC 2004	74.51
RRII 105	62.02
CD(P=0.05)	12.43

Table 4. Accession. Potential accessions showing dry rubber yield

Sl. No	Accessions	Dry rubber yield ($gt^{-1}t^{-1}$)	% of the yield of RRII 105
1	AC 166	65.26	101.90
2	RO 2385	61.13	95.45
3	AC 2004	54.87	85.68
4	RO 2908	54.59	85.24
5	AC 692	46.71	72.94
6	AC 650	46.73	73.00
7	AC 655	44.62	70.00
8	AC 635	43.97	68.66
9	MT 922	42.45	66.29
10	AC 638	41.46	64.58
11	RO 368	40.73	63.60
12	AC 668	36.25	63.34
13	MT 1020	35.09	54.79
14	AC 3013	32.74	51.02
15	RO 2976	32.27	50.00
	RRII 105	64.04	
	CD(P=0.05)	20.06	

Table 5. Potential accessions for timber yield

Sl.No	Accession	Timber yield (m^3)
1	MT 941	0.16
2	RO 1347	0.14
3	MT 199	0.14
4	MT 1630	0.14
5	AC 661	0.14
	RRII 105	0.08
	CD(P= 0.05)	0.05

922) showed resistance to *Phytophthora* with 95% leaf retention.

Second FET was planted during 2003 where 22 accessions are being evaluated. In this, RO 2629 (68.42 cm) recorded the highest girth followed by AC 4149 (58.78 cm) and AC 626 (52.99 cm). RO 2629, AC 4149 and AC 716 recorded highest yield in the second year of tapping. In FET 2008, among 26

wild accessions, accession AC 176, MT 77 and RO 2846 recorded good girth. In FET 2010, 13 wild accessions, selected from SBN 2004 having more than 80 percent test tap yield of RRII 105 are under evaluation.

Various studies undertaken so far could identify several accessions that were potentially superior for individual secondary traits in comparison to the check clone RRII 105. Since the highest yielder RRII 105 is not a very vigourous clone, a cut-off of 125% of its girth has been fixed for identifying desirable wild accessions for girth. The accessions showing superiority for individual traits can be used in hybridization with elite cultivars for yield enhancement if confirmed in further evaluation (Table 6).

Table 6. Accessions showing superiority for individual traits

Character	Number of accessions
Dry rubber yield	55
Girth	215
Number of latex vessel rows	29
Bark thickness	41

On-Farm/ Participatory evaluation:

One wild accession AC 166 which gave mature yield on par with check clone RRII 105, is advanced to On-farm trials at five locations *viz.*, B.C. Cheruvally estate, Erumely, Malankara estate, Thodupuzha, Mooply estate, Trissur, Calicut estate, Kozhikode and Bethany estate, Kanyakumari for evaluating the performance along with the three selected IRCA clones (IRCA 130, IRCA 111, IRCA 109). This is the first wild accession advanced to a participatory clone evaluation trial.

Screening for timber characteristics

a. Field screening

Annual girth, monthly yield and timber volume were estimated among 19 wild accessions and six Wickham clones. At the age of 11 years, two accessions (MT 941 & AC 650) had the timber (bole) volume significantly higher than that of PB 260, RRII 33, RRII 118, RRII 105 and RRIM 600 (Table 7). Two Acre accessions (AC 685 and AC 707) showed yield statistically on par with that of RRII 105 & PB 235 (Table 8).

Table 7. Tree girth and bole volume

Wild Accessions	Girth (cm)	Bole volume(m3)	Wild Accessions	Girth (cm)	Bole volume(m3)
MT 922	64.53	0.09	RO 322	51.34	0.08
MT 941	61.44	0.10	AC 655	48.92	0.05
MT 915	58.45	0.07	MT 935	48.36	0.04
AC 650	57.49	0.13	MT 1020	48.09	0.04
RO 255	56.60	0.05	AC 707	43.06	0.04
MT 919	56.01	0.07	AC 685	41.97	0.04
AC 635	54.39	0.06	AC 637	41.77	0.05
MT 1032	53.67	0.06	AC 651	41.23	0.06
MT 999	51.67	0.06	RO 879	36.33	0.03
AC 1021	51.13	0.05	CD(P=0.05)	14.46	0.03
Domesticated Clones					
RRII 118	68.03	0.08			
PB 235	66.98	0.05			
PB 260	65.41	0.08			
RRII 33	63.66	0.06			
RRII 105	56.88	0.06			
RRIM 600	53.55	0.06			

Table 8. Dry rubber yield

Wild Accessions	Dry rubber yield ($gt^{-1}t^{-1}$)	Wild Accessions	Dry rubber yield ($gt^{-1}t^{-1}$)
AC 685	28.29	MT 941	3.39
AC 707	30.34	MT 935	2.97
RO 255	13.75	RO 322	2.22
AC 635	9.44	AC 651	1.37
AC 1021	5.31	MT 999	2.66
MT 922	6.97	AC 655	2.20
MT 915	6.28	AC 637	0.68
MT 1032	9.21	AC 650	3.02
MT 919	3.72	RO 879	0.18
MT 1020	2.91		
Domesticated Clones		CD(P=0.05)	15.67
RRII 105	38.69		
RRIM 600	33.57		

Wild Accessions	Dry rubber yield ($gt^{-1}t^{-1}$)	Wild Accessions	Dry rubber yield ($gt^{-1}t^{-1}$)
PB 235	30.53		
RRII 33	29.98		
RRII 118	23.89		
PB 260	20.49		

b. Screening for timber quality traits through lignin biosynthesis studies

Preliminary studies have been conducted for the early detection of wood quality through lignin biosynthesis studies. The study revealed that the lignin precursor enzyme, CAD (Cinnamyl Alcohol Dehydrogenase) activity is positively correlated with lignification in *Hevea* (Reghu, 2007). The estimation and quantification of lignin bio-polymer and Cell wall phenolics (CWP) was also carried out in 19 wild accessions and 6 Wickham clones from the timber evaluation trial (Table 9). Four wild accessions showed significantly higher percentage of wood lignin than the controls. One accession, RO 879 (0.46 μ OD/ mg of Dry weight of EXR) had significantly higher CWP than that of 6 Wickham clones. In comparison with the controls (Wickham clones) the percentage of CWP was increased in 12 wild accessions and decreased in 3 accessions.

Table 9. Percentage of cell wall phenolics and lignin in wild accessions

Wild Accessions	Cell Wall Phenolics (m OD/ mg/dry wt. Of EXR)	PB 235 (0.310)	RRII 33 (0.295)	RRII 105 (0.304)	RRII 118 (0.307)	RRIM 600 (0.319)	PB 260 (0.359)	Lignin (%)
MT1021	0.372	+20.8	+25.9	+22.3	+21.3	+16.6	+3.6	21.43
MT1020	0.368	+18.4	+24.4	+20.9	+19.0	+15.2	+2.4	21.98
MT 935	0.362	+16.5	+22.5	+19.0	17.9	+13.4	+1.0	21.04
MT 922	0.321	+3.4	+8.7	+5.6	4.6	+0.5	-11.0	23.33
MT 919	0.349	+12.6	+18.3	+14.8	13.8	+9.4	- 2.7	20.34
MT 999	0.294	-5.3	-0.5	-3.3	4.1	-8.0	-18.0	19.28
MT 1032	0.279	-10.0	-5.4	-8.0	8.9	-12.4	-22.1	20.77
MT 915	0.318	+2.6	+7.8	+4.8	3.8	-0.2	-11.3	20.12
MT 941	0.276	-11.3	-6.7	-9.4	10.2	-13.7	-23.3	22.73
AC 635	0.297	-4.3	+0.6	-2.3	3.1	-6.9	-17.2	22.52
RO 322	0.252	-19.2	-15.0	-17.5	18.2	-21.4	-30.4	23.99

Wild Accessions	Cell Wall Phenolics (m OD/ mg/dry wt. Of EXR)	PB 235 (0.310)	RRII 33 (0.295)	RRII 105 (0.304)	RRII 118 (0.307)	RRIM 600 (0.319)	PB 260 (0.359)	Lignin (%)
RO 255	0.338	+8.8	+14.4	+11.2	10.2	+5.9	-5.8	23.90
AC 685	0.339	+9.2	+14.8	+11.6	10.6	+6.3	-5.6	24.75
RO 879	0.460	+48.1	+55.7	+51.3	49.9	+44.1	+28.1	21.50
AC 637	0.289	-6.7	-1.9	-4.7	-5.6	-9.2	-19.3	20.50
AC 650	0.374	+20.4	+26.6	+23.0	+21.9	+17.2	+4.2	21.03
AC 655	0.329	+5.9	+11.4	+8.2	+7.2	+3.1	-8.4	20.27
AC 651	0.341	+9.8	+15.5	+12.2	+11.2	+6.9	-5.0	19.32
AC 707	0.347	+11.7	+17.5	+14.1	+13.1	+8.7	-3.4	20.22
Wickham Clones								
PB 235	0.310							20.84
RRII 33	0.295							22.73
RRII 105	0.304							22.77
RRII 118	0.307							23.36
RRIM 600	0.319							20.08
PB 260	0.359							21.97

Screening for stress tolerance

Screening for biotic stress tolerance

Field screening of 3561 wild genotypes in six conservation nurseries for *Oidium* resistance over a period of three years revealed that 140 accessions had relatively higher resistance (< 25 % disease incidence), while two accessions had less than 10% disease incidence. Three rounds of laboratory screening of all the wild accessions resulted into the identification of 152 and 281 accessions that showed consistent tolerance to *Phytophthora* and *Corynespora* respectively. (Table 10) gives

Table 10. Distribution of disease tolerant accessions among the different provenances.

Provenance	*Oidium*	*Corynespora*	*Phytophthora*
Acre	-	112	32
Rondonia	2	134	16
Mato Grosso	-	35	99
Others	-	-	5
Total	2	281	152

the distribution of accessions among the different provenances, showing tolerance to various diseases.

As part of *Corynespora* disease tolerance screening of wild *Hevea* accessions, a set of 51 shorted listed accessions along with 2 control clones were multiplied for confirmation in a hotspot evaluation trial and was planted in a statistically laid out design at Rubber Board Regional Nursery, Ulickal, Iritty, (a hot spot region) in collaboration with Plant Pathology division.

Screening for abiotic stress tolerance

Drought tolerance

For identifying the drought tolerance potential of this germplasm collection, various studies were undertaken in different sets of this material, using various drought tolerance attributing characters. Screening was conducted in each set with an objective to identify the potential accessions possessing the drought tolerance attribute used for that study. As occurrence of drought condition is an increasing problem even in traditional rubber growing belt of India, studies conducted in the traditional regions of India find scope to identify the potential accessions. Studies were also attempted in the actual hot-spot regions of India experiencing severe drought condition, which are helpful for developing location specific clones suitable for growing in those regions. A summary of various studies so far conducted in India both in the traditional as well as non-traditional belts with an aim to identify potential wild accessions possessing one or more character contributing towards drought tolerance are mentioned below:

(a) Cellular membrane stability

A preliminary screening was carried out in 100 wild accessions using cellular membrane stability as a parameter for screening in the first phase and in the second phase out of 10 selected accessions, detailed studies were conducted using various drought related morphological, anatomical, physiological and biochemical characters. Analysis of data on the 10 selections indicated significant genotypic differences for growth and leaf structural characters. From this set of 100 accessions MT 41, MT 55 and AC 650 were identified as accessions possessing drought tolerance characters (Mercy, 2000).

(b) Chlorophyll content, Epicuticular wax and Cell membrane injury

Another study conducted was in a set of 37 accessions where biochemical

parameters such as chlorophyll content, chlorophyll stability, epicuticular wax content and injury to cell membrane were the parameters used for screening. Here also significant genotypic difference was clear for all the parameters studied indicating the scope for selection in this valuable collection. Accessions RO 5163, MT 5090 and AC 4939 exhibited high chlorophyll content whereas chlorophyll stability was more in MT 5156 and MT 5098. Accessions RO 4595 and RO 4620 showed high epicuticular wax content which intern gives them the capacity to preserve the moisture content (Mercy, 2005).

(c) Pot culture study

A pot culture experiment was conducted using 16 wild accessions for identifying the potential drought tolerant accessions. Data recording on morphological and physiological parameters related to drought tolerance was conducted during the pre and post summer periods and anatomical observations on primary xylem points and intra xylary phloem were studied using twig samples. Plant height, girth, number of whorls, number of leaves, internodal length and petiole length were the morphological parameters studied and afternoon leaf water potential after withholding irrigation was used as a drought related physiological parameter. Morphological characterization based on drought sensitive parameters such as leaf size, appearance of leaf surface, presence or absence of wax coating on leaf surface, leaf yellowing and senescence and vigor of plant was done and mortality rate among the accessions was assessed for identifying the potential drought tolerant accessions. The clonal response of these accessions to drought stress was assessed to identify the potential drought tolerant wild accessions. Wide variability was observed among the accessions for all the characters studied. MT 1623 showed superiority in growth and vigour and high values for intra xylary phloem (Table 11). In general, leaf water potential was high in MT accessions. From this set, the accessions MT 1681 and MT 1623 were identified as the potential ones for further detailed field evaluation towards developing a drought tolerant *Hevea* clone (Mercy, 2010).

(d) Growth parameters

Ten accessions were selected based on preliminary studies conducted among 450 accessions for growth parameters during summer months. In this selected accessions, studies were conducted both in the field and in a glasshouse to assess genotypic difference for growth and vigour under water stress. The parameters

Table 11. Potential accessions for structural traits along with twig diameter

Accession/Clone	No. of proto xylem points (PXP)	No. of intra xylary phloem points (IXP)	Diameter of twig (mm)	IXP/ mm diameter of twig
MT 1623	104.50	77.00	15.60	4.94
MT 200	81.80	75.38	20.30	1.82
MT 3714	74.25	59.25	17.90	3.31
MT 48	54.67	38.79	12.80	3.03
Tjir 1	60.83	30.67	12.50	2.45
RRII 105	48.60	38.20	9.00	4.24
RRII 208	56.00	39.88	11.90	3.35
RRIM 600	57.48	49.15	7.70	6.38
V.R.	3.24**	3.71**	7.32**	
CD	30.15	30.40	3.88	
CV	26.53	42.04	15.08	

** Significant at 1%

recorded in the field experiment include scion height (cm), basal diameter of scion at 20 cm from bud union (mm), number of leaves, number of leaf flushes, interflush distance (cm), single leaflet area (cm^2) and specific leaf weight (SLW) ($mg.cm^{-2}$). In the glass house, at the end of two months water stress, morphological observations such as basal diameter of scion (mm), fresh weight of the scion (g), dry weight of the scion (g) and Dry Matter Stress Tolerance Index (DMSI) were worked out.

Here also, the results revealed existence of wide genetic variability among the wild germplasm accessions for various growth parameters such as scion height (cm), scion basal diameter (mm), number of leaves, number of leaf flushes and single leaflet area (cm^2) in the early growth phase. Dry matter production and dry matter stress tolerance index also gave an indication towards the stress tolerance capacity of the wild accessions. Three accessions MT 41, MT 76 and MT 66 showed general superiority irrespective of their growth conditions. These parameters contributing to general vigor of an accession in the early growth could be employed for screening the large number of germplasm materials for drought tolerance (Mercy, 2006).

(e) Leaf anatomical parameters

Another attempt made using wild accessions for identifying their drought tolerance potential was use of leaf anatomical parameters such as thickness and number of cells in unit length of paliside tissue, thickness of mesophyll tissue, leaf lamina thickness and leaf vein diameter. Among this, palisade cell number per unit length was found to play an important role towards imparting drought tolerance and significant genotypic difference was evident indicating the scope for selection among the wild accessions (Mercy, 2004).

(f) Intrinsic drought tolerance

Another attempt for screening this material for drought tolerance was that made by Crop Physiology group of RRII. Using physiological tools, phenotypes with intrinsic drought tolerance traits were identified. For this, visual scoring of drought symptoms such as leaf yellowing and senescence was done in the germplasm source bush nurseries during summer months. Out of 3772 accessions screened, 165 showed intrinsic drought tolerance. Ten top ranking accessions from different source bush nurseries were subjected to laboratory screening using chlorophyll fluorescence technique. Based on the principle that an intrinsically drought tolerant accession will be showing small reduction in photochemical efficiency of Photosystem II, accessions with drought tolerance potential could be identified. Accessions MT 4856, MT 5100, MT 5078 and MT 4788 were the potential accessions whereas accessions MT 4694 and RO 4615 were the drought susceptible accessions. Among these four potential accessions, MT 5078 and MT 5100 were proved to be the most intrinsically drought tolerant ones after the detailed nursery evaluation (Nair, 2005).

A hot spot screening of wild *Hevea* germplasm was initiated in 1996 at the Regional Research Station, Sukma in the Bastar region of Central-Eastern India, Chattishgadh State. This region experiences high temperature along with high light intensity during summer months and rain fall is concentrated around 4 months. A total of thirty wild accessions of Acre, Mato Grosso and Rondonia provenances and six Wickham clones were screened for drought tolerance in this trial. Highly significant clonal variability was observed for all the characters studied. Among the thirty wild accessions RO 5430 (58.67 cm) and RO 2635 (58.67 cm) recorded the highest girth. Bole volume ranged from 0.045 m^3 (AC 623) to 0.133 m^3 (RO 5430). The wild accession RO 5363 (20.79 g/t/t) recorded

yield on par with RRII 105 (19.58 g/t/t). The accessions RO 5363 and RO 5430 were found to be superior in this drought prone region which could be of use in future crop improvement programmes (Rao *et al.,* 2006).

Wild germplasm accessions were also taken in batches to grow in the Regional Research Station (RRS), Dapchari located in the North Konkan region of Maharashtra state in India, a region experiencing high temperature (exceeding 40° C in April- May), high light intensity and very low soil moisture during the summer months with a rainfall pattern confined mainly to four months in a year, where, the average annual rain fall is 7.5mm per day. The objective was to know the growth performance of the wild accessions and to select those which could tolerate three to four years of drought condition at this location. First set of 63 accessions were grown there during the year 2001 and after exposing the plants to three years of drought stress there, eight MT accessions could be identified (Table 12) as having drought tolerance potential and from the second set of 42 accessions grown there, six potential accessions could be identified (Mercy, 2009).

During 2003, third set comprising 130 accessions were evaluated and from this batch seven potential drought tolerant accessions were identified. In this set, two accessions RO 1769 and RO 2976 gave good test tap yield compared with the check clone RRII 105.

From the above mentioned evaluation studies conducted both at traditional and non traditional regions; around 25 potential accessions could be identified for breeding programmes. These accessions are now at detailed evaluation in the hot spot region of Dapchari, where the actual drought condition prevails. The results so far indicated the outstanding performance of the accessions MT 4788 and MT 4856 of which the former is a proven good yielder in the traditional region. If this accession continues to perform well in the drought prone region with good yield, it will be a promising one in the breeding programme for developing drought tolerant *Hevea* clone.

Cold tolerance

Cold temperature is increasingly becoming limiting factors for the growth and productivity of plants especially in the non-traditional rubber growing areas (Jacob *et al.,* 1999). 1981 IRRDB wild *Hevea* collection is a rich source of genes conferring tolerance to abiotic stresses such as cold and heat. Breeding for cold

Table 12. List of wild accessions selected for detailed field evaluation

Set No.	Accession	Drought tolerant attribute
I	MT 54	Good height, girth, number of whorls, RWC and stable performance
	MT 1579	Good growth even after experiencing stress
	MT 67	Good growth (height and girth), RWC
	MT 1668	No. of leaves, good growth (height and girth) and girth increment
	MT 1616	No. of leaves, no. of whorls, RWC, good growth (height and girth)
	MT 1627	No. of leaves, no. of whorls, RWC, good growth (height and girth)
	MT 1649	Good growth even after experiencing stress
	MT 80	Good growth increment and RWC even after experiencing stress
II	MT 41	Good foliar production, good RWC, higher rate of girth increment
	RO 1248	Good growth (height and girth), good RWC in post drought period
	AC 765	Good growth (height and girth, no. of whorls and leaves)
	RO 2524	Good growth (height and girth, no. of whorls)
	MT 945	Good growth (height and girth, no. of leaves)
	MT 58	Good growth (height and girth, no. of leaves)

tolerance is one of the thrust areas of research in the non-traditional rubber growing region of North-East India. A total of 64 wild accessions were evaluated for cold tolerance in two trials at Regional Experiment Station, Nagrakata, West Bengal. Monthly yield and girth of the thirteen-year-old accessions recorded during pre- and post- winter period, showed significant variation. Higher annual girth was observed in RO 2902, MT 5105, MT 915, RO 2727, MT 900 and RO 2387 as compared to check clones SCATC 93/114 and RRIM 600. Accessions AC 471, AC 3514, AC 4653and RO 2908 are the potential yielders of these two trials at Nagrakata indicating their cold tolerance.

Utilization of wild Hevea germplasm in breeding programmes

Even though there are relatively few direct selections for yield, utilization of the wild germplasm is possible only through hybridization of wild accessions possessing the desirable alleles, with elite cultivars. Though most of the progeny

of such W x A crosses are expected to be poor yielders, recombination between such diverse parents could result in new genotypes with high yield as well as the desired secondary traits. Even if the yield of a recombinant with the desired wild allele falls just short of acceptable levels, backcrossing once to the elite parent or to another high yielder will definitely improve the chances of recovering a desirable new genotype in the next generation. Similar superior wild accessions have been incorporated as parents in Wickham x Amazonian hybridization programs in other rubber research Institutes also. In India, W x A crosses were attempted in 1989, 1997, 2002, 2007 and 2009. The 43 clones that evolved from the 1989 programme incorporating eight wild accessions were planted in two small-scale trials in 1995. 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/JP/3/6 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 6th 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. 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. Their performance in the fourth year of growth and testtap yield was evaluated. Four accessions with yield levels ranging from 17.2 - 35.7 g/t/10 tappings were superior to RRII 105 which had a yield of 15.06 g/t/10 tappings. Six other accessions also had relatively high yields ranging from 9.95 to 14.63 g/t/10 tappings. The girth of these clones ranged from 11.79 to 18.37 cm, while that of the control was 8 cm.

HP programmes with wild drought tolerant accessions and potential domesticated clones were also initiated in 2007 with the objective of developing high yielding drought tolerant *Hevea* clones. The cross RRII 429 x RO 1769 resulted in four successful progenies, which are under evaluation. Hybrid plants resulted from the HP 2009 programme are under field evaluation for their early growth performance.

Establishment of an arboretum of entire wild germplasm collection at North-east is also under way. This will allow the plants to grow up to maturity as this is not possible in SBNs. Collection of open pollinated seeds (WxA and AxA) is also possible from this arboretum which can be subjected to rigourous screening for desirable natural recombinants. The first set of 95 wild accessions is multiplied and a polybag nursery is raised at RRS, Tura, Meghalaya for field planting during next year. More OP gardens are also envisaged as and when desirable wild accessions are identified. However, there is a long way to go for realizing the expected benefits from such a large collection of wild germplasm.

Conclusions

India maintains 4548 Wild *Hevea* germplasm in *ex situ* conservatories in traditional and non-traditional regions since 1980s. Characterization and evaluation of these potential accessions at multidisciplinary level are progressing in a phased manner. The challenge before the breeder lies in evolving strategies for identification of the desired variability from the available genetic resources. Wild *Hevea* germplasm is definitely a source of abundant variability required for development of location specific clones capable of withstanding different constraints prevalent especially in non traditional regions. 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 the speedy and reliable selection process. 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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