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ABSTRACT

Crop improvement programmes in the Rubber Research Institute of India have been of the cyclical generation-wise assortative mating kind since the beginnings of *Hevea* breeding in India in 1954 until the 1980s. The realization of the dangers of gene erosion and the need for broadening the genetic base of *Hevea* led India to join the IRRDB in collection, characterization and conservation of a share of the 1981 IRRDB wild *Hevea* germplasm from Brazil. India's share of this wild Amazonian germplasm was introduced into the country between 1984 and 1990 in batches and is being conserved at two locations , one in the traditional region in Kerala, and the other in the non traditional region of North East India.

India now maintains 4548 wild *Hevea* germplasm accessions from Acre, Rondonia and Mato Grosso provenances in *ex situ* conservation nurseries. Characterisation and evaluation of these valuable accessions have been progressing in a phased manner since the mid 1980s. As expected, wide variability in morphological, anatomical, physiological and biochemical attributes contributing to rubber yield, timber yield and quality, growth and secondary characters has been evident from evaluations done in phases by a multidisciplinary team of scientists of the RRII. Being a potential repository of genes that could impart tolerance/ resistance to biotic and abiotic stresses that the rubber tree is exposed to in the traditional and non- traditional regions of rubber cultivation in India, the RRII has been relentlessly pursuing measures for proper conservation , evaluation and above all, utilization of this precious germplasm for systematic and planned genetic improvement programmes. Early results from the

studies done at RRII have been promising. The W x A hybridization programmes effected since 1990 have resulted in some heterotic hybrids that have surpassed the popular high yielding clones in rubber and timber yield. Though few in number, there are indications of direct selections as well, for yield, from this wild germplasm.

The promising accessions, along with the heterotic hybrids and present day Wickham clones could, over the next one or two generations unleash tremendous genetic variability which would be a boon to any *Hevea* breeder in the pursuit of bridging the gap between theoretical and realized yields in *Hevea brasiliensis*. Gene mining, marker assisted selection and proper and timely utilization of results could help take *Hevea braslìensis* to newer environs and underutilized land in a country like India and help increase the production of rubber, a strategic raw material for which an acute shortage is predicted.

This status report details the efforts underway and results obtained by the Rubber Research Institute of India with regard to the conservation, evaluation and utilization of the 1981 IRRDB wild *Hevea* germplasm collection.

Key words: Conservation, Characterization, Evaluation, *Hevea braslìensis*, India, IRRDB, Molecular markers, Utilization, W x A crosses, Wild germplasm.

1. Introduction

The primary center of diversity of *Hevea* spp. is the Amazon basin in Brazil, situated within 5^0 latitudes at altitudes below 200 m. The climate of this region is equatorial monsoon type characterized by mean monthly temperature of 25 to 28^0 C , well distributed rainfall with no marked dry period (Strahler, 1969; Bradshaw, 1977).Under commercial cultivation, rubber trees perform best in climates closely resembling that of its original habitat. Only a few regions in India meet the requirements. Fortunately, rubber can grow and evolve successfully under moderately deviating conditions also (Vijayakumar *et al.*, 2000).Generations of *Hevea* breeding by selection and hybridization have resulted in India becoming one of the secondary centres of diversity of this species, as in the case of Malaysia, Indonesia, Sri Lanka and other major rubber growing countries. But the pedigree of present day cultivated clones in India can be traced back to only a handful of Wickham clones , an indicator of insufficient variability in the breeding pool. Directional breeding towards high rubber yields, cyclical generation-wise assortative mating (GAM) and monoculture of only the best yielding clones propagated by budding, have had a major role in the further narrowing of genetic variability. Nevertheless, considering the out crossing nature of this perennial species with heterozygozity being the rule, untapped genetic variability among the Wickham clones still exists.

Genetic variability being the backbone of crop improvement, the timely action taken by the IRRDB in 1981, in collaboration with EMBRAPA of Brazil, to collect and conserve the fast diminishing genetic resources of *Hevea* from the Amazon forests, resulted in a huge quantity of germplasm collected from the Brazilian states of Acre (AC), Rondonia (RO) and Mato Grosso (MT). The material consisted of 64723 seeds and budwood of 194 high yielding trees (ortets) (IRRDB, 1982), which was shared between IRRDB and Brazil. IRRDB member countries received their shares from the African and Malaysian distribution centers in phases over the ensuing years. India has also received a share of this fresh germplasm, which is being conserved, characterized, evaluated and utilized with the objective of broadening the gene pool for further genetic advance and also for achieving yield stability and sustainability. India's share of this wild Amazonian germplasm was introduced into the country between 1984 and 1990 in batches and is being conserved at two locations , one in the traditional region in Kerala, and the other in the non traditional region of North East

India. India is thus poised to reap the benefits of having an array of germplasm from the primary and secondary centres of diversity, for which tremendous amount of painstaking effort is required by generations of plant breeders in the years ahead.

2. Conservation

On receipt of the budwood every year from Malaysia in the 1980s, the first step was to multiply and conserve the genotypes in nurseries. Each genotype was assigned a new accession number. The plants were spaced at 1 x 1 m, with each accession being represented by 5-16 plants, along with selected popular Wickham clones as checks. 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. The remaining plants in each accession were cut back after the 2nd or 3rd year and converted to source bushes to provide budwood. The 13 primary source bush nurseries have been listed by Varghese *et al.*, 2002.

A major portion of the material sent to the North East was lost due to the unfavorable conditions. In the traditional region too, some accessions are lost every year, reducing the number of accessions available in the conservation-cum- source bush nurseries (SBNs). The present status in India is shown in Table 1.

Table 1. The 1981 IRRDB wild *Hevea* germplasm conserved in 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 attrition in the nurseries every year has prompted us to start a programme of re establishment of the wild germplasm in new nurseries. The programme was initiated in 2003, and completed over a period of six years, with one set of accessions being multiplied and planted each year. The accessions have been planted at a spacing of 1 x 1 m, in an augmented RBD, with four checks, and a plot size of five plants, so that the data collected in the juvenile phase can be statistically analysed. These plants will be cut back after data on three years of growth has been collected.

3. Characterization

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; Rao *et al.*, 2005a; Varghese *et al.*, 2002) . The newly re-established SBNs are ideal for characterization in the juvenile phase as they provide uniform sets of accessions in terms of age. Morphological characterization on qualitative traits is being carried out in these nurseries in the first year of growth. All the wild accessions will therefore be characterized in the juvenile stage. However, since these nurseries have been planted at close spacing and will be cut back after 3 years, only those accessions that go on to the full-fledged evaluation trials can be characterized in the mature phase. So far, a total of 3620 accessions have been characterized in the juvenile stage using 22 morphological traits.

3.2. Molecular characterization

The applications of molecular markers in germplasm management include assessment of genetic diversity, identifying duplicates, forming core collections, and for Marker Assisted Selection of potential parents for crop improvement programmes. Studies have been conducted at RRII for cataloguing the genetic diversity among genotypes from different sources.

Clustering of a set of 60 wild accessions using 22 RAPD primers showed that genetic diversity in this collection is closely related to the geographical provenance of the accessions. Another study involving RAPD profiling of 110 wild accessions using 16 informative primers indicated considerable genetic variation among the genotypes from Acre, Rondonia and Mato Grosso provenances (Varghese *et al.*, 2002b; Abraham *et al.*, 2005). The genotypes were grouped into eight distinct clusters, which showed geographical distinctiveness to the three states from where these material were originally collected. A few accessions which were distinctly divergent in the clusters were also identified.

3.3. Characterization for biochemical components of yield

Some of the biochemical parameters like latex ATP which has a high positive correlation with rubber yield along with sucrose and thiols were successfully used for evaluating the yield potential of 14 selected wild *Hevea* germplasm lines . Biochemical parameters in latex (ATP, sucrose and thiols) were estimated in eight high yielding and five low yielding accessions along with the check clone RRII 105. Among them eight accessions (AC 166, MT 1020, MT 179, RO 2629, AC 675, RO 2385, AC 2004, AC 655) exhibited potential for high yield and six accessions (AC 655, AC 707, AC 637, AC 158, AC 661 and AC 162) were of low yield potential based on the biochemical characterization (Table. 2). Among the accessions, AC 166 and AC 2004 showed high ATP content in latex similar to RRII 105. Thiol and sucrose content in latex was also comparable in RRII 105 and AC 2004.

Table 2: Biochemical parameters in the latex of different germplasm accessions

Accessions	Yield (g/t/t)	ATP (µM)	Sucrose (mM)	Thiols (mM)
AC 166	53.4±9.1	265.9±7.1	10.8±2.6	0.126±0.003
MT 1020	36.3±4.8	175.9±4.2	7.9±0.86	0.120±0.009
MT 179	31.6±8.8	183.1±12.3	13.9±2.2	0.146±0.021
RO 2629	44.9±1.3	230.8±16.8	9.4±0.45	0.129±0.014
AC 675	32.5±2.5	191.5±5.02	12.3±2.3	0.138±0.015
RO 2385	26.1±5.6	241.6±7.5	13.9±2.5	0.197±0.045
AC 2004	35.7±4.6	270.8±3.9	8.8±0.6	0.160±0.024
AC 655	37.6±8.7	197.8±6.9	11.2±1.1	0.135±0.021
AC 707	4.8±1.0	128.8±3.85	11.3±1.8	0.131±0.012
AC 637	8.5±1.2	110.0±3.7	24.2±4.4	0.159±0.032
AC 158	5.6±1.41	150.8±7.2	16.0±0.7	0.116±0.001
AC 661	7.2±2.6	137.9±3.5	6.1±0.22	0.103±0.011
AC 162	4.7±0.73	147.9±7.5	19.1±2.8	0.374±0.051
RRII 105	64.9±3.8	263.2±5.6	7.7±1.1	0.170±0.024

3.4. Genetic studies on wild *Hevea* germplasm using molecular markers

3.4.1. Genetic relationships and evolution

Genetic relationships among the wild *Hevea* germplasm along with cultivated clones were assessed using RAPD and microsatellite/SSR markers generated in the genome lab of RRII for (1) evaluation of genetic divergence within and between the wild *Hevea* genotypes from Acre, Rondonia and Mato Grosso provenances of Brazil and their evolutionary relationship with cultivated clones, (2) identification of specific DNA profiles for individual wild genotypes, which have considerable value in documentation of the identity of the genetic resources available in the collection and (3) identification of duplicates to manage and make rational use of genetic resources.

Our studies showed existence of considerable genetic variation (30 - 60%) among the wild genotypes from three different provenances: Acre, Rondonia and Mato Grosso of Brazil. A minimum genetic distance of 55% was noticed between provenances, with Acre and Rondonia genotypes being genetically closer than the Mato Grosso genotypes. Genetic relatedness of Mato Grosso accessions with cultivated *Hevea* clones was evident .

Another related study was performed with a set of universal chloroplast microsatellite markers for getting insights of the chloroplast genome differentiation among the wild *Hevea* genotypes. Analysis of the chloroplast genome provides information that is complementary to that obtained from the nuclear genome on the population dynamics of the species. Ten consensus chloroplast microsatellites were used to study the relationships among the wild genotypes from three different provenances. Out of 10 primer pairs for chloroplast microsatellites: ccmp1 to ccmp10, five primer pairs gave amplification in *Hevea* (ccmp2, ccmp3, ccmp5, ccmp6 and ccmp7). When these five markers were tested with six wild germplasm (two each from the three different provinces Acre, Rondonia and Mato-Grosso), polymorphisms were detected only with the primer pair for ccmp6, which amplified an intergenic region (ORF 77-ORF 82) of other plant species. Other chloroplast microsatellite markers were monomorphic in wild *Hevea*. The chloroplast marker ccmp6 appeared to be highly informative as it detected six different haplotypes among the wild germplasm when tested with the same set of 60 genotypes. These polymorphisms were always due to a variable number of mononucleotide residues within A/T stretches in the amplified regions.

Based on this marker, Acre genotypes could be discriminated from the genotypes originating from other two provenances, as most of the Acre genotypes showed the presence of a novel haplotype of the chloroplast genome (size 94 nt). The number and distribution of haplotypes in different regions suggest that *Hevea* has been independently evolved in several locations. This chloroplast marker could be used to address certain questions regarding its geographical origin.

3.4.2. Allele discovery

Dinucleotide repeats were detected at 3'UTR of 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGR*) gene encoding HMG-CoA reductase in *Hevea brasiliensis*, an important enzyme involved in rubber biosynthesis. Existence of two microsatellite alleles and their repeat compositions was demonstrated earlier in cultivated rubber clones. Both alleles contained perfect poly $(AG)_n$ repeats interrupted by a short sequence of 12 nucleotides and allelic variation at this microsatellite locus was the result of repeat length polymorphisms. In wild populations of rubber, nine microsatellite alleles ('A' to 'I') were identified at the *HMGR* locus revealing a wide allelic diversity compared to cultivated clones. Out of nine, four alleles ('B', 'C', 'D' and 'G') were present in higher frequencies than the others. In total, 15 allelic combinations were noticed for *HMGR* among wild accessions and four of them were unique. Twenty-five out of 60 wild accessions were found to be homozygous for the above four alleles ('BB', 'CC', 'DD' and 'GG') and the rest were heterozygous, characterized by 11 different allelic combinations. Repeat-length polymorphisms were noticed in these four alleles prevailing among wild *Hevea* accessions. This work is also a significant step towards understanding the functional variability of *HMGR* for latex production in *Hevea brasiliensis*.

3.4.3. Single Nucleotide Polymorphisms (SNPs)

SNPs are rapidly becoming the marker of choice for many applications in genome analysis due to their abundance in the genome. Four representative wild *Hevea* accessions from each of the three different provenances Acre, Mato Grosso and Rondonia were used in SNP detection. Twelve genes were amplified at their 3' untranslated region (3'UTR) and sequenced. In total 30 SNPs and one indel were detected in four of these 12 genes at their 3'UTR and associated coding sequences. SNPs detected in the Mato Grosso genotypes showed similarity with popular clones. While analyzing SNPs in these genes, we could observe clear discrimination of the wild accessions provenance-wise indicating geographic distinctness of the wild accessions. This observation supports our earlier findings on genetic relationship using RAPDs and microsatellites. Effective haplotypes were predicted which could be used as geographical markers for wild accessions.

4. Evaluation

Germplasm evaluation is the most crucial step towards their utilization in crop improvement programmes. The perennial nature of the crop, the spacing required and the large number of accessions involved, makes it impossible to evaluate the entire wild germplasm at a time. A phased manner of evaluation is thus adopted in India, planting one set of accessions every year based on the availability of land. All the wild accessions are subjected to a preliminary agronomic evaluation. Selected accessions from the preliminary evaluations are then subjected to detailed evaluation in Further Evaluation Trials (FETs).

4.1. Preliminary evaluation trials (PET)

4.1.1. Early strategy

During the first decade after import of the accessions (up to 2002), the strategy had been to evaluate the wild germplasm in a phased manner by taking random samples of accessions into Preliminary Evaluation Trials (PETs) for studying their performance under field conditions for various agro-morphological and anatomical characters. The design and spacing of these trials varied from simple lattice at close spacing to augmented RBDs at normal spacing, with suitable check clones, so as to accommodate a maximum number of genotypes at a time. The ortets, being preliminary selections from the forests and fewer in number, are evaluated in an RBD or lattice design. So far a total of 1341 accessions have been established in 17 preliminary evaluation trials in five different stations in the traditional and non-traditional rubber growing areas (Rao *et al.*, 2008).

Analysis of data from the various trials, as well as preliminary observations on sets of accessions from the nurseries, has revealed wide variability among the genotypes for most of the agro-morphologic traits, bark structural characters and juvenile yield indicating scope for selection of accessions with desirable characters (Annamma *et al.*, 1988,1989; Abraham *et al.*, 1992; Mercy *et al.*, 1992,1993; Madhavan *et al.*, 1993; Reghu *et al.*, 1996; Rao *et al.*, 1996). Test tap yield showed the highest variability. In general, the wild genotypes recorded poor yield, as anticipated, when compared to the domesticated clones. However, a few promising genotypes were identified with respect to yield. A total of 132 wild accessions have been identified from various preliminary studies so far. Rao *et al.*, 2008 have listed 46 accessions that were among top yielders in various PETs and SBNs. These accessions will be evaluated in detail in statistically laid out further evaluation trials to confirm their yield potential. Accessions that were potentially superior for individual secondary traits in comparison to the check clone RRII 105 include 215 nos. for girth, 29 for number of latex vessel rows and 41 for bark thickness (Rao *et al.*, 2008).

4.1.2. Present modified strategy

The strategy of preliminary evaluation of all accessions has been modified in view of the increasing constraints on land and manpower . Instead of systematic evaluation of the accessions in field trials up to the mature stage, the preliminary evaluation is carried out in the newly re established conservation nurseries, which have been laid out in augmented RBDs with four check clones, a plot size of five and a spacing of 1m x 1m. Juvenile characterization and growth data are collected in the first three years of growth, before the nurseries are cut back for budwood. One round of test tapping of all accessions in the second year of growth and a second round for selected accessions in the third year are being conducted to identify potential high yielders. Variability for juvenile quantitative growth traits in a set of 544 wild accessions of re-established nursery 2003 has been reported by Rao *et al.*, 2008.

Genetic divergence studies in wild germplasm assume great importance in the context of their utilization in hybridization programs. Genetic diversity was estimated using the k-means statistic for seven quantitative traits in a set of 544 accessions,(Rao *et al.*, 2005b) forming 15 clusters as shown in Table 3 .

Table 3. Clustering of a set of 544 wild accessions

Cluster No.	No. of accessions	Acre	Mato Grosso	Rondonia	Others (7 series)
I	5	3	-	2	-
II	49	12	10	27	-
III	74	37	13	24	-
IV	1	1	-	-	-
V	67	21	15	30	1
VI	1	1	-	-	-

VII	34	15	4	15	-
VIII	31	7	6	16	2
IX	20	4	2	14	-
X	73	45	8	19	1
XI	31	5	4	22	-
XII	2	2	-	-	-
XIII	33	13	5	15	-
XIV	61	35	9	14	3
XV	62	21	10	31	-
Total	544	222	86	229	7

The Shannon-Weaver Diversity indices (SDI) estimated for the 22 morphological qualitative traits during characterization of 195 accessions, also showed high levels of diversity for most traits showed in Table 4.

Table 4. SD indices for a set of 195 accessions.

Descriptor	SDI	Descriptor	SDI
Height	0.8591	Petiolule orientation	0.6851
Girth	0.5815	Leaf blade colour	0.1416
Axillary bud	0.1944	Lustre	0.7586
Leaf scar	0.0454	Texture	0.0454
Leaf storey shape	0.6891	Leaves shape	0.4209
Separation of whorls	0.7485	Margin	0.9999
External appearance	0.4196	Leaves size	0.4441
Leaves-pulvinous	0.2350	Cross sectional appearance	0.5413
Petiole shape	0.7319	Leaf apex	0.5110
Size	0.8038	Colour of veins	0.1944
Angle orientation	0.4447	Leaf blade surface	0.8751

According the current strategy, accessions that consistently perform well in comparison with the check clones in the re-established nurseries are evaluated in full-fledged field trials. Those accessions showing 80% or more of the yield level of the check clones are planted in large scale trials, while those with 50-80% of the yield of the checks are first being evaluated in clonal nurseries, and the potential ones are advanced to further evaluation trial or used in hybridization programmes to infuse new alleles into the gene pool. In spite of the apparent drawbacks of this method (a sample size of only 5, close spacing and test tapping in two seasons), this will be a much faster way to screen all the accessions for yield. Test tapping of the first two sets of accessions totaling 1563 nos. planted in 2003 and 2004, has already identified a total of 59 potential yielders with yield level of more than 50% of the respective checks. Of these, 28 are very promising with yield levels of at least 80% of that of the check clone RRII 105. These accessions are now under further evaluation in three different trials planted in 2003, 2005 and 2010. A clonal nursery evaluation of a set of 16 accessions with 50 – 80 % test tap yield selected from source bush nursery 2004 was laid out during 2010. 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.

4.2. Further evaluation trials (FET)

Detailed further evaluation of the 1981 IRRDB wild germplasm accessions selected in the preliminary evaluation trials is in progress. The first FET was planted at RRS, Padiyoor during 1995 with RRII 105 as control, consisting of 80 wild genotypes comprising 21 Acre, 18 Rondonia and 41 Mato Grosso genotypes. These accessions had originally been selected on the basis of preliminary information on yield, girth and number of latex vessel rows in the initial years of germplasm evaluation. The design adopted was a simple lattice design with a plot size of four and four replications, at normal spacing. These genotypes in terms of growth and yield were evaluated at the age of 13 years. The trial was opened for regular tapping in S/2 D3 system in 2004. Monthly dry rubber yield was recorded and the annual yield from the 2^{nd} year to the 6^{th} year of tapping,studied.

After 11 years of growth, six accessions viz. MT 54 , MT 191, MT 1032, RO 2385,MT 1674 and RO 1517 were superior in girth to the check clone, RRII 105 (49.18 cm) and 73 accessions had girth comparable with that of RRII 105. The mean dry rubber yield ranged from 1.00 g/t/t (AC 637) to 45.75 g/t/t (MT 179) whereas the check clone RRII 105 recorded 48.25 g/t/t. Eleven accessions (AC- 6 nos.; RO-3 nos.; MT-8 nos.) had comparable yield with the check. Thirteen accessions showed 80 % - 95 % of the yield of the check and 34 accessions showed 50% - 79% of the yield of RRII 105. Ten accessions (AC-2 nos.; RO-1 no.; MT-7 nos.) had significantly higher bole volume than RRII 105 of which 4 accessions showed promising yield as well (52% - 89% of the check clone). The dry rubber yield was analyzed in both summer and peak seasons separately and the total yield was computed over five years of tapping. With regard to total yield , though 10 accessions showed yield on par with RRII 105 in the 4th year, only one accession (AC 166) showed this trend in the 5th year of tapping (Table 5).

Table 5. Wild accessions showing promising performance for dry rubber yield (g/t/t)

Accns.	Summer yield		Peak yield		Total yield	
	4th year	5th year	4th year	5th year	4th year	5th year
AC 166	31.64	46.56	57.06	66.83	49.40	56.69
MT 1020	27.96	18.49	50.97	37.40	48.68	27.94
RO 2908	25.96	16.87	56.99	56.25	44.45	44.38
MT 54	22.40	15.48	41.04	30.76	35.35	20.26
RO 2629	22.21	19.44	37.73	30.88	33.13	25.14
RO 2385	21.81	15.20	49.01	39.71	41.12	32.57
AC 2004	21.32	23.09	47.34	46.09	39.44	34.59
AC 675	20.55	14.29	49.88	36.82	43.44	27.11
AC 670	18.95	28.43	27.77	53.01	26.99	40.72
AC 685	13.10	22.60	22.46	43.51	19.57	33.06
RRII 105	23.44	41.39	44.51	83.16	45.79	62.27
CD (P=0.05)	9.10		19.44		13.23	

Six accessions (AC-3 nos.; MT-3 nos.) continued to show significantly higher bole volume than RRII 105 of which one Acre accession (AC 650) also showed better performance for yield (64% of the yield of RRII 105). In terms of bole volume, 59 accessions were statistically on par with RRII 105.The evaluation of these was continued in the 15th year also (6th year of tapping).

As part of the biochemical analysis, ATP content was recorded in two seasons (peak & stress seasons) in selected high yielding and low yielding accessions. The accession AC 166 had the highest dry rubber yield (58.78 g/t/t) which is statistically on par with the yield of RRII 105 (64.74 g/t/t) after 6th year of tapping.. This accession consistently showed high dry rubber yield for the last two years also (4th & 5th year of tapping) and had 91% of the yield of the check clone, RRII 105 with high ATP content during the peak and stress seasons. Considering the consistent yield performance, the accession AC 166 has been advanced to On-Farm evaluation trial in five locations under different environments. Two more FETs comprising a total of 45 wild accessions and 4 controls have been planted in 2003 and 2005 in simple lattice designs with 4 replications and a plot size of 4. Test tapping of FET 2003 has indicated that most of the selections were on par with RRII 105, while some had very high yield.

4.3 Core collection

Core collection refers to a sub set of germplasm accessions identified so as to represent the total genetic variability present in the entire germplasm. The large size of the 1981 IRRDB collection has necessitated the identification and formation of core subsets for a more effective evaluation and utilization of this collection. This can be based on data on genetic distance between the accessions based on morphological and molecular markers, in the absence of which, a cruder method of random stratified sampling of accessions based on their provenance is possible. The formation of a core collection from this large base collection was initiated at RRII using quantitative data on a set of 80 wild accessions, from which a representative subset of 23 accessions was identified. This was validated using Shannon Diversity indices for another set of qualitative data on the same set of accessions . The analysis has to be repeated in other sets of accessions till the entire germplasm can be represented by a smaller and more manageable number of accessions, which nevertheless retains the diversity of the original set. This will be the logical first set of accessions to be searched for desirable alleles for finding donors of various characters for use in hybridization.

5. Screening for timber characteristics

5.1.Screening for timber volume

Nineteen wild accessions selected from the source bush nursery based on growth performance were planted in a field trial in 2002 along with six Wickham clones as controls to evaluate their timber characteristics. The performance of these accessions in terms of bole height, tree girth, bole volume, percentage of wood lignin and cell wall phenolics was analyzed. Wide variation was noticed for girth and bole height in the wild accessions and Wickham clones (Table 6). At the age of 9 years among the wild accessions, the girth of the trees ranged from 34.26 cm (AC 651) to 3.21(MT 919).Among the six Wickham clones, PB 260 showed the highest girth (58 cm). Eleven accessions were statistically on par with PB 260 and PB 235 for girth. One wild accession AC 650 had bole volume equal to that of the check clone PB 235. Among the wild accessions, the bole height was maximum for AC 650

(4.75 m). MT 999 had the highest girth, which was statistically on par with RRII 33, the highest among the Wickham clones.

Table 6. Annual girth and bole volume at the age of 9 years

Sl.No.	Accessions	Girth(cm)	Bole volume (m^3)
1	MT 919	53.21	0.04
2	MT 941	52.08	0.04
3	AC 685	52.33	0.02
4	MT 999	49.18	0.04
5	RO 255	49.41	0.03
6	MT 915	48.33	0.04
7	MT 935	47.64	0.03
8	MT 1032	47.92	0.03
9	RO 322	47.23	0.04
10	MT 922	45.87	0.04
11	AC 707	46.99	0.02
12	AC 635	42.44	0.02
13	AC 650	42.33	0.05
14	AC 655	42.23	0.02
15	AC 1021	39.64	0.03
16	MT 1020	36.80	0.03
17	RO 879	35.79	0.02
18	AC 637	35.41	0.02
19	AC 651	34.26	0.02
Wickham Clones			
1	PB 260	58.77	0.04
2	PB 235	58.00	0.05
3	RRII 118	54.41	0.05
4	RRII 33	51.54	0.04
5	RRII 105	49.34	0.03
6	RRIM 600	45.95	0.03
CD (P= 0.05)		14. 56	0.02

5.2. Screening for timber quality through lignin biosynthesis studies.

Screening of *Hevea* germplasm for timber quality traits through lignin biosynthesisis studies is in progress. The lignin content was estimated in 19 wild accessions and 6 Wickham clones. Lignin content ranged from 19.28 - 24.75% among the wild accessions whereas in Wickham clones, the range was 20.08 - 23.36%.Among the wild accessions, AC 685 (24.75%) recorded the maximum lignin content and MT 999 (19.28%) the minimum. Among the six Wickham clones, lignin content was the highest in RRII 118 (23.36%) followed by

RRII 105 (22.77%), RRII 33 (22.73%) and minimum for RRIM 600 (20.08%). Three wild accessions (AC 685, RO 322 & RO 255) showed higher lignin content than clone RRII 118. Four accessions (AC 685, RO 322, RO 255 & MT 922) had higher lignin content than RRII 105 & RRII 33.Nine accessions had higher lignin compared to PB 260, PB 235 & RRIM 600 (Reghu *et al.*, 2005).

6. Screening of wild *Hevea* germplasm accessions against biotic stresses

Abnormal leaf fall and shoot rot caused by *Phytophthora* sp., powdery mildew caused by *Oidium heveae*., and leaf diseases due to infection by *Colletotrichum* spp. and *Corynespora cassiicola* comprise the major diseases that affect rubber plantations in India (Edathil *et al.*,2000), apart from Pink disease caused by *Corticium salmonicolor*.

While screening for leaf diseases can be undertaken in the immature stage and by laboratory assay, the study of tolerance levels to stem and root diseases requires large scale field experimentation, which is worthwhile only in the case of accessions which show some promise in terms of agronomic attributes, when land and manpower are the constraints. There are no known sources of resistance to *Oidium* in the cultivated Wickham clones, while certain clones like Fx 516 and RRII 33 which have shown high levels of tolerance to *Phytophthora* are poor in terms of rubber yield. The wild germplasm is a repository of genes that could confer tolerance to such biotic stresses and intensive efforts were made in the RRII at screening the accessions received as India's share of the 1981 IRRDB collection from the Malaysian centre.

6.1. Sources of resistance to *Phytophthora*

6.1.1. Field screening for abnormal leaf fall disease

Abnormal leaf fall disease incidence was assessed on trees maintained in the Source Bush Nurseries for 2691 accessions from Mato Grosso (MT) Acre (AC) and Rondonia (RO) provenances. Per cent leaf retention was assessed by visual scoring and the details are given in Table 7.

Table 7: Abnormal leaf fall disease - Percent leaf retention in wild germplasm accessions

No of accessions and their % leaf retention				Total no.
>75 %	50-75 %	25-50 %	< 25 %	
257	269	198	1967	2691

6.1.2. Laboratory screening against *Phytophthora*.

The accessions which gave more than 50% leaf retention in the field screening and accessions which had no trees in the field were screened for *Phytophthora* resistance in the laboratory by detached leaf disc technique. Leaf discs were inoculated with zoospore suspensions and the lesions size was measured 72 h after inoculation. RRII 105,a tolerant clone and RRIM 600 a highly susceptible clone to *Phytophthora* were kept as control. The selected accessions from the preliminary screening were further subjected to two more rounds of inoculation and screening for confirming their tolerance/resistance. The accessions which showed consistently tolerant reaction in all the rounds were selected after the third round and the data was statistically analyzed. Eighteen accessions were significantly better than RRII 105 and 86 were on par with RRII 105 (Table 8) .

Table 8: The number of promising *Phytophthora* tolerant wild germplasm accessions

No. of accessions	Acre (AC)	Mato Grosso (MT)	Rondonia (RO)	Total No.
Better than RRII 105	3	14	1	18
Equal to RRII 105	12	65	9	86
Total	15	79	10	104

The 18 accessions which are better than RRII 105, a *Phytophthora* tolerant clone hold promise as sources of resistance to the pathogen. These include the Rondonia genotype RO 4423, three Acre genotypes AC 2016, AC 3146 and AC 462 and 14 Mato Grosso genotypes MT 1617, MT 4494, MT 1631, MT 4436, MT 1581, MT 1715, MT 2219, MT 2233, MT 3707, MT 4252,MT 1027, MT 900, MT 4702 and MT 4874.

6.1.3. Field screening of germplasm accessions for shoot rot disease

3617 accessions in the source bush nursery were screened for shoot rot incidence and all accessions were found susceptible to the disease.

6.2. Sources of resistance to *Oidium*

6.2.1. Field screening of germplasm accessions against powdery mildew disease

Field screening of 3561 wild genotypes in six conservation nurseries for Oidium resistance was carried out on five plants per accession employing a 0 – 5 scale and per cent disease intensity was calculated. The screening done over a period of three years revealed that 140 accessions showed relatively higher resistance (< 25 % disease incidence) in the preliminary screening, while two accessions had less than 10% disease incidence for three consecutive years, indicating tolerance/ resistance to the pathogen.

6.2.2. Screening of wild germplasm against powdery mildew in North East India

Powdery mildew being the only serious disease of rubber in North East India, screening of wild germplasm for tolerance to *Oidium* was done at the Regional Research Stations of the RRII in the states of Tripura and Assam situated in North East India.

Based on 6 years' screening on wild *Hevea* germplasm accessions conserved at Taranagar Research Farm, Tripura, a total of 21 wild accessions were found to be moderately tolerant to powdery mildew disease (24.8 to 32% PDI). Three wild accessions (*viz.* MT 5136, RO 5160 and RO 5087) out of 21 short-listed *Hevea* germplasm seems to be more tolerant to powdery mildew disease. Another batch of 87 accessions were screened and four viz RO 3794, RO 5055, RO 5365 and MT 4859 were observed moderately tolerant to powdery mildew disease recording 20-22 percent disease intensity. These accessions showed similar results for three consecutive years of 2008, 2009 and 2010.

Screening for powdery mildew disease on wild accessions of *Hevea* germplasm was carried out in a germplasm conservation garden at Sarutari Research Farm in Assam for five years. Based on five years' screening, a total of 17 accessions were found to be moderately tolerant to powdery mildew disease (20 to 32%). Severity of powdery mildew disease was assessed again on the 17 short-listed accessions following staggered cutting method for confirmation. Three wild accessions *viz.* RO1737, AC 587 and AC 5302 seem to be exhibit stable tolerance to powdery mildew disease over 3 years (Table 9).

Table 9. Incidence of powdery mildew disease on wild accessions of *Hevea* at Sarutari

Sl. Nos.	Wild accessions	Powdery mildew disease in different years (PDI)			Mean of 3 years
		2008-09	2009-10	2010-11	
1	MT 5150	36.2	36.0	40.0	37.4

2	MT 4706	30.0	40.0	36.0	35.4
3	MT 5101	34.2	50.0	30.0	38.1
4	MT 4854	37.0	40.0	20.0	32.4
5	MT 4768	39.0	50.0	36.0	41.7
6	MT 4895	37.6	40.0	40.0	39.2
7	MT 4729	39.0	34.0	40.0	37.7
8	RO 1737	20.0	20.0	20.0	20.0*
9	RO 5047	30.0	Dried	-	-
10	RO 4607	34.0	34.0	Dried	-
11	RO 5250	37.6	36.0	40.0	37.9
12	RO 6157	28.0	30.0	32.0	30.0
13	RO 4627	20.0	28.0	30.0	26.0
14	AC 587	26.0	20.0	20.0	22.0*
15	AC 5302	20.0	28.0	20.0	22.7*
16	AC 4673	30.0	36.0	20.0	28.7
17	AC 6174	27.0	32.0	24.0	31.0

6.3. Sources of resistance to *Colletotrichum*.

6.3.1.Field screening of germplasm accessions against Colletotrichum leaf disease

Field screening of germplasm accessions in the SBN (2003) for Colletotricum leaf disease incidence at CES, Chethackal was carried out. Disease assessment in 1452 accession was carried out by visual scoring using the following 0-5 scale:

0 - no disease ,

1- very few spots on very few leaves,

2- few spots on many leaves,

3 – many spots on many leaves,

4 – Heavy spotting curling of leaves & tip drying on many leaves,

5 – Heavy spotting and leaf fall, more than half of the area drying.

The results are given in Table 10.

Table 10: Grading of wild accessions for Colletotrichum leaf disease

Grade	No. of accessions
0	161
1	251
2	103
3	74
4	156
5	707
Total	1452

Screening of accessions with low disease incidence (0 -1 grade) has to be repeated for confirming their tolerance.

6.4. Sources of resistance to *Corynespora*

6.4.1. Laboratory screening for resistance to Corynespora leaf disease

Accessions were screened against *Corynespora. cassiicola* in the laboratory by detached leaf technique. Leaves were inoculated with spore suspensions and the lesion size was measured 72 h after inoculation. GT 1, a tolerant clone and RRII 105 a highly susceptible clone were kept as control. The 600 accessions selected from preliminary screening were subjected to a second round of screening and 416 were short listed. Final round of screening is being continued.

7. Screening of wild *Hevea* germplasm accessions against abiotic stresses

7.1. Screening for drought tolerance :

Environmental stresses such as drought, temperature extremes, low or high light intensity etc. are becoming major limiting factors for growth and productivity of rubber plants in the given vagaries of global climate change in India and the world over. This has been reported (Jacob and Satheesh,2010 a and b) from both the traditional and non-traditional regions of rubber cultivation in India. The large number of accessions from the IRRDB collection of wild germplasm available with the RRII are genetically divergent and having been evolved in the tropical conditions prevailing in Brazil, it can be assumed that some of them might possess useful genetic traits that impart tolerance to stresses like drought, high light, high temperature etc. An effort was made by the Crop Physiology group of RRII for screening these germplasm lines with suitable physiological tools to identify phenotypes with intrinsic drought tolerance traits. Field level screening of the wild accessions in the drought and cold prone areas in Dapchari (Maharashtra), Sukma (Chhattisgarh) and in the sub-Himalayan region at Nagrakatta (Northern part of W.Bengal) respectively have also been initiated.

The first attempt was to identify the most intrinsically drought tolerant plants from the large pool of wild accessions by visual empirical scoring of few drought related traits. The study was carried out in one year old plants grown at Central Experiment Station of RRII.

7.1.1. Field scoring and selection for intrinsic drought tolerance

Field scoring for drought tolerance traits was carried out in source bush nurseries (SBN) of wild germplasm accessions by empirical scoring of certain drought tolerance traits during summer months (February-April). The photosynthetic pigments in the leaves of *Hevea* normally get photooxidized and the colour of the leaves turn yellow during summer months. During this period the soil and atmospheric drought coupled with high solar radiation prevailing is maximum in the field. Under this severe stress condition in the field, plants usually shed their leaves and the extent of leaf senescence occurring will depend on their intrinsic tolerance capacity. Taking these two important characters into consideration, visual scoring of drought symptoms such as leaf yellowing and leaf senescence was carried out in germplasm lines in the field during summer.

The plants exhibiting less yellowing and delayed senescence were ranked as the the most intrinsically drought tolerant ones . Out of the 3772 accessions screened by field scoring, 165 accessions showed intrinsic drought tolerance potential (Table 11). The accessions studied were sorted numerically and ranked according to the percent leaf senescence and leaf yellowing separately. Based on an empirical scoring, ten genotypes each from the six source bush nurseries were selected (Table 12).

Table 11: Number of wild accessions from the IRRDB collection selected from source bush nurseries (SBN) for their intrinsic drought tolerance traits.

Source bush nursery	Total no. of accessions screened	No. of accns. with intrinsic drought tolerance
SBN 2003	588	70
SBN 2004	976	49
SBN 2005	700	16
SBN 2006	806	11
SBN 2007	500	12
SBN 2008	202	7
Total	3772	165

Table 12: Top ranking 10 wild accessions selected by field scoring for drought tolerance traits from different germplasm nurseries.

Sl. no.	GERMPLASM SOURCE BUSH NURSERY (SBN)					
	SBN 2003	SBN 2004	SBN 2005	SBN 2006	SBN 2007	SBN 2008
1	MT 47	RO 855	RO 2634	RO 2990	AC 1963	RO 3160
2	MT 62	MT 1673	MT 2210	AC 513	AC 4138	RO 2806
3	AC 4280	AC 3146	RO 1406	RO 1398	AC 3822	RO 1370
4	RO 4393	RO 2864	RO 1413	RO 2723	AC 1914	AC 3325
5	AC 4006	AC 612	RO 1420	RO 1528	AC 4072	AC 1846
6	AC 4286	MT 944	RO 1421	RO 2907	RO 3672	AC 1969
7	AC 3825	MT 196	AC 173	AC 513	AC 460	RO 3223
8	MT 3687	MT 1652	RO 3184	RO 1398	AC 3567	-
9	RO 2838	AC 2009	MT 2231	RO 2723	AC 482	-
10	AC 2206	AC 1751	RO 1425	RO 1528	AC 4159	-

7.1.2. Laboratory screening of wild germplasm for intrinsic drought tolerance

Intrinsic drought tolerance capacity of wild germplasm was evaluated in laboratory using a simple, rapid and non-invasive technique. The chlorophyll fluorescence technique has long been used extensively as an effective biophysical tool to monitor the response of plants to various stresses. A reduction in maximum photochemical efficiency of Photosystem II is often taken as a good indicator of photo-damage to photosynthetic apparatus in plants that are under stress. A *Hevea* genotype that is intrinsically tolerant to drought will exhibit relatively small inhibitions compared to susceptible ones when exposed to a stress.

Leaf samples collected from field and the leaf discs (2.0 cm size) were subjected to *in-vitro* drought stress in laboratory using 60% polyethylene glycol (PEG) solution and simultaneously exposed to light (350 μ mols m^{-2} s^{-1}). A similar set of samples was maintained in distilled water in dark without imposing water stress, to measure the maximum potential quantum yield of PS II in dark-adapted state. The photochemical activity of leaf was measured using a Pulse Amplitude Modulated Fluorometer (PAM- 2000, Waltz, Germany). The maximum potential photochemical activity of PS II in dark adapted state (dark Fv/Fm) was measured, then the samples were exposed to an actinic light of 120 μmols m^{-2} s^{-1} and the effective quantum yield of PS II (Q.Y) and the efficiency of excitation energy capture by open PS II (Fv'/Fm') were measured at a constant fluorescence emission in the steady state. Percent reduction in PS II activity due to *in-vitro* drought stress was estimated and the genotypes were ranked for the intrinsic tolerance traits separately for Fv/Fm, Q.Y. and Fv'/Fm'. The genotypes that consistently appeared in the top ten ranks for all three parameters were identified as the most tolerant ones (Table 13) as reported by Nair *et al.*, 2005.

Table 13: Ranking of germplasm accessions (top ten) based on percent inhibitions in dark Fv/Fm, quantum yield and Fv'/Fm'.

Rank. No.	Fv/Fm	Quantum Yield	Fv'/Fm'
1	MT 4713	MT 4859	MT 4787
2	RO 4580	RO 4913	AC 4833
3	MT 4856	AC 4689	MT 4772
4	MT 5100	MT 4788	MT 5152
5	MT 4772	MT 4856	MT 4856
6	MT 5078	MT 4756	MT 5100
7	MT 4802	MT 5078	MT 4713
8	MT 4788	MT 5100	MT 4788
9	MT 5152	MT 5089	AC 4689
10	MT 5114	MT 4747	MT 5078

Out of 182 accessions screened in the laboratory, four Mato Grasso (MT) accessions that exhibited less reduction in PS II activity under *in-vitro* drought and light stresses, were selected as the ones most tolerant to drought stress. The reduction was found to be very low in accessions like MT 4713, MT 4856, MT 5078 etc. Similarly in light (fv'/fm') the inhibition was low in accessions MT 4856, MT 5100 and MT 5152. A low PS II quantum yield was observed in genotypes MT 4859, RO 4913 and MT 4788. Among the wild accessions screened, four MT accessions viz. MT 4856, MT 5100, MT 5078 and MT 4788 were found to be intrinsically tolerant to drought stress. Two accessions viz., MT 4694 and RO 4615 were identified as most susceptible ones, for further studies.

7.1.3. Nursery evaluation of wild germplasm for intrinsic drought tolerance traits:

The four wild accessions found tolerant in *in-vitro* drought assay using the laboratory technique were further evaluated for drought tolerance at nursery stage for various physiological and biochemical traits. Germplasm accessions MT 5100, MT 5078, MT 4788 and MT 4856 along with the known drought tolerant and drought susceptible clones, RRIM 600 and RRII 105, respectively, were evaluated in polybags for water deficit stress by withholding irrigation for duration of three weeks. PS II photochemical efficiency (both in dark and light), leaf chlorophyll content, leaf water potential and total soluble sugar content of leaves were estimated in stressed and unstressed plants.

Table 14: Maximum photochemical efficiency (dark Fv/Fm) and PS II quantum yield in leaves of wild germplasm accessions exposed to drought stress.

Sl. no	Clones		Dark Fv/Fm	PSII Quantum yield
1	MT 4856	C	0.81 ± 0.009	0.59 ± 0.016
		S	0.74 ± 0.005	0.39 ± 0.011
2	MT 4788	C	0.79 ± 0.005	0.48 ± 0.017
		S	0.722 ± 0.004	0.40 ± 0.015
3	MT 5078	C	0.80 ± 0.007	0.56 ± 0.012
		S	0.76 ± 0.005	0.48 ± 0.014
4	MT 5100	C	0.79 ± 0.003	0.56 ± 0.021
		S	0.74 ± 0.004	0.44 ± 0.035
5	RRIM 600	C	0.79 ± 0.005	0.53 ± 0.035
		S	0.74 ± 0.01	0.44 ± 0.011
6	RO 4615	C	0.81 ± 0.006	0.62 ± 0.014
		S	0.71 ± 0.005	0.37 ± 0.011
7	MT 4694	C	0.79 ± 0.005	0.58 ± 0.017
		S	0.73 ± 0.017	0.39 ± 0.045
8	RRII 105	C	0.80 ± 0.007	0.59 ± 0.011
		S	0.73 ± 0.01	0.35 ± 0.033

Compared to dark Fv/Fm significant differences were observed in PS II quantum yield of plants exposed to drought and light. Two accessions, MT 5078 and MT 5100, and control clone (RRIM 600) maintained better photochemical activity under drought condition

(Table 14). Leaves of clone RRIM 600 and accession MT 5100 maintained higher mid-day leaf water potential compared to other clones (Table 15). The total chlorophyll content and total soluble sugar content in leaves of stressed plants were reduced significantly due to drought stress (Tables 16 and 17). Clone RRIM 600 showed higher sugar content in leaves followed by MT 5078 under drought. A comparative evaluation of the drought tolerance capacity of germplasm accessions was made based on their percent reduction in various physiological parameters in drought compared to fully irrigated plants (Table 18).

Table 15: Leaf water potential of wild germplasm accessions exposed to drought stress.

Sl. No	Clones		Mid-day leaf water potential (- bar)
1	MT 4856	C	22.92 ± 0.48
		S	29.4 ± 0.78
2	MT 4788	C	22.86 ± 0.53
		S	28.84 ± 0.93
3	MT 5078	C	23.2 ± 0.29
		S	26.96 ± 1.16
4	MT 5100	C	24.24 ± 0.31
		S	28.34 ± 0.74
5	RRIM 600	C	23.46 ± 0.58
		S	27.08 ± 1.10
6	RO 4615	C	23.62 ± 1.03
		S	28.92 ± 0.92
7	MT 4694	C	23.74 ± 0.50
		S	30.2 ± 0.65
8	RRII 105	C	24.24 ± 0.86
		S	30.34 ± 0.40

Table 16: Chlorophyll content in leaves of wild germplasm accessions exposed to drought stress.

Sl. no.	Clones		Total chlorophyll	Chlorophyll *a*	Chlorophyll *b*
1	MT 4856	C	3.60 ± 0.10	2.91 ± 0.11	0.69 ± 0.03
		S	2.94 ± 0.07	2.37 ± 0.05	0.56 ± 0.02
2	MT 4788	C	2.97 ± 0.11	2.27 ± 0.09	0.70 ± 0.02
		S	2.40 ± 0.13	1.91 ± 0.10	0.44 ± 0.03
3	MT 5078	C	3.43 ± 0.14	2.70 ± 0.11	0.72 ± 0.03
		S	3.02 ± 0.14	2.43 ± 0.12	0.59 ± 0.01
4	MT 5100	C	3.31 ± 0.16	2.59 ± 0.13	0.72 ± 0.04
		S	2.95 ± 0.12	2.37 ± 0.10	0.58 ± 0.01

5	RRIM 600	C	3.12 ± 0.16	2.48 ± 0.13	0.64 ± 0.02
		S	2.86 ± 0.03	2.28 ± 0.03	0.58 ± 0.01
6	RO 4615	C	3.63 ± 0.12	2.80 ± 0.08	0.82 ± 0.05
		S	3.13 ± 0.05	2.49 ± 0.10	0.63 ± 0.01
7	MT 4694	C	3.63 ± 0.25	2.86 ± 0.18	0.76 ± 0.07
		S	2.85 ± 0.07	2.22 ± 0.06	0.63 ± 0.01
8	RRII 105	C	3.67 ± 0.09	2.83 ± 0.09	0.84 ± 0.02
		S	2.78 ± 0.09	2.16 ± 0.08	0.62 ± 0.01

Table 17: Total soluble sugar content in leaves of wild germplasm accessions exposed to drought stress.

Sl. No	Clones		Total sugar content (mg/g f.w't)
1	MT 4856	C	58.76 ± 5.35
		S	36.03 ± 1.31
2	MT 4788	C	48.91 ± 4.67
		S	42.44 ± 3.81
3	MT 5078	C	50.02 ± 4.40
		S	44.42 ± 3.02
4	MT 5100	C	46.28 ± 3.86
		S	42.99 ± 1.47
5	RRIM 600	C	51.50 ± 3.46
		S	46.14 ± 3.60
6	RO 4615	C	44.33 ± 2.36
		S	38.39 ± 2.48
7	MT 4694	C	44.64 ± 1.7
		S	35.95 ± 1.37
8	RRII 105	C	52.76 ± 3.32
		S	38.13 ± 1.84

In conclusion, accessions MT 4856, MT 4694, RO 4615 and clone RRII 105 exhibited higher degree of inhibition compared to the check clone RRIM 600 and the germplasm accessions MT 5078, MT 5100 and MT 4788. Among the germplasm accessions, MT 5078 and MT 5100 emerged as the most intrinsically tolerant lines from the drought tolerance assay using nursery plants. The selected germplasm lines with intrinsic stress tolerance traits are useful genetic materials and can be made available for more detailed investigation on the fundamental physiological and molecular level investigations. Accessions MT 5078 and MT 5100 thus emerge as potential donor parents for drought tolerance breeding programs.

Table 18: Percent reduction in various drought tolerance traits in germplasm accessions exposed to drought stress.

S. no.	Clones	Chlorophyll content	Sugar content	Dark Fv/Fm	PS II Q.Y.	Water potential	Percent total
1	MT 4856	18.33	38.68	8.24	33.36	28.24	126.85
2	MT 4788	19.11	13.22	8.43	10.93	26.31	78.05
3	MT 5078	11.95	11.11	5.34	13.67	16.1	58.97
4	MT 5100	10.87	7.10	6.05	22.05	16.98	63.05
5	RRIM 600	8.32	10.40	5.7	16.92	15.42	56.74
6	RO 4615	13.77	13.42	11.40	39.36	22.4	100.35
7	MT 4694	21.48	19.46	7.5	32.47	27.3	108.24
8	RRII 105	24.25	27.65	8.77	41.24	25.2	127.11

7.1.4. Genetic variability for drought tolerance parameters

Drought stress is common even in traditional areas and this occurs concomitantly with high temperature and high light during summer. A preliminary screening was carried out in 100 wild genotypes in the traditional region in Kerala 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. Parameters such as girth increment and rate of stomatal conductance during stress period, dry matter stress tolerance index, chlorophyll fluorescence, chlorophyll and leaf epicuticular wax content, thickness of palisade layer in the leaf and number of palisade cells per unit length were considered to be among the reliable indices for screening genotypes for drought tolerance. Analyses of data on the 10 selections indicated significant genotypic differences for all these growth and leaf structural characters. Each wild genotype was ranked based on parametric relationships with drought tolerance. The genotype MT 41 scored the highest rank sum followed by MT 55 and AC 650 which indicate the potential of these genotypes for drought tolerance.

Another set of 37 accessions was screened for drought tolerance potential using biochemical parameters such as total chlorophyll content, chlorophyll stability, epicuticular wax content and injury to cell membrane. The data revealed significant variation among the accessions for all the parameters studied (Table 19).

Table 19: Chlorophyll content, chlorophyll reduction and wax content of top 5 accessions

Total Chlorophyll content (mg cm-2)	Chlorophyll reduction (%)	Epicuticular wax content (µg cm-2)
RO 5163 (4.6)	MT 5156 (2.7)	RO 4595 (183.6)
MT 5090 (4.6)	MT 5098 (3.2)	RO 4620 (165.0)
AC 4939 (4.5)	MT 4740 (6.9)	MT 5100 (154.3)
MT 4878 (3.9)	MT 5131 (6.4)	MT 5098 (151.7)
RO 5162(3.9)	RO 4595 (6.9)	MT 5141 (147.7)
RRII 105 (3.6)	RRII 105 (13.9)	RRII 105 (122.6)
RRIM 600 (3.0)	RRIM 600 (8.3)	RRIM 600 (71.0)
Variance Ratio = 4.53**	Variance Ratio = 6.8**	Variance Ratio = 19.34**

7.1.5. Hot spot screening in drought prone areas

Field trials were also taken up for screening the wild germplasm accessions in drought prone areas in the non traditional regions. At the Regional Research Station of the RRII located in Dapchari, a drought prone tract in Maharashtra state, 235 wild accessions were screened and 24 promising ones could be identified. In general Mato Grosso and Rondonian accessions were superior for drought tolerance. Accessions RO 1769 and RO 2976 showed superiority with respect to juvenile yield under drought stress. The preliminary selections with potential for yield and drought tolerance are now being subjected to further detailed evaluation in a new trial initiated at RRS, Dapchari. Another batch of accessions selected by field scoring at nursery level have now been planted for hot spot screening under field conditions in the non-traditional drought prone area, Dapchari, Maharashtra state, in India.. Twenty four wild germplasm accessions planted in the field were scored for leaf yellowing and senescence during hot summer months. Seven wild germplasm lines *viz.* MT 1627, MT 1623, MT 43, MT 1668, MT 1681, RO 2387 and RO 2153 were identified as the most intrinsic tolerant accessions in the drought prone area.

Another set of 30 accessions evaluated in a dry region in another Regional Research Station of the RRII located in Sukma, Chattisgarh state, along with six Wickham clones, revealed three wild accessions, RO 5363, RO 5430 and RO 5329 to be promising (Krishan *et al.*, 2010).

7.2. Screening for cold tolerance

7.2.1. Conservation and evaluation of wild *Hevea* germplasm in North East India

Apart from south- western peninsular India, rubber cultivation has now expanded to the cold prone North eastern states and drought prone regions of Central India where soil conditions are suitable for tree growth. A portion of the wild germplasm collection (972 accessions) is being maintained in conservation nurseries in the Taranagar Farm of the Regional Research Station of RRII at Agartala, situated in the state of Tripura and in the Sarutari Farm under the Regional Research Station of RRII in Assam, North East India (Varghese et al., 2002).

Over 247 accessions from the 1981 IRRDB germplasm collection were planted in a gene pool garden at the Research Farm, in Taranagar, Agartala, Tripura in 1989. During the course of time, nearly 60 accessions failed to survive the climatic constraints like low temperature during winter, strong winds and seasonal soil moisture stress experienced in this North eastern state. At present, 187 accessions are conserved in the garden.

Out of 600 germplasm accessions established at Sarutari Farm in Assam, 457 survived the extreme climatic conditions of cold winters followed by high light stress experienced in the region and these are being conserved *in situ*.

7.2.2. Evaluations in Tripura State

The wild *Hevea* germplasm evaluation experiments were conducted with 87 selected wild Brazilian accessions, which included 48 Mato Grosso, 34 Rondonia and 5 Acre accessions. RRIM 600, the popular clone, cultivated extensively in North East India, was included as the check clone. The trial was laid out following Simple Lattice Design at 3 x 3m spacing. Recommended cultural practices were followed and trees were opened for tapping by the 9^{th} year after planting.

A wide range of variation was exhibited by the accessions, for parameters like girth , yield and bole volume (Table 20). The girth of AC 5502 was consistently highest both at the time of opening as well as three years after tapping and was higher than that of the check clone RRIM 600.Many of the accessions like AC 5502 (79.6 cm), MT 4906 (74.7 cm), MT 5136 (74.6 cm), MT 4713 (73.4 cm) exhibited higher girth than RRIM 600 at the time of opening.

The accessions showed wide variation in their yield performance too. All the accessions exhibited lower yield compared to the popular high yielding check clone RRIM 600. The accession AC 5502 that showed highest girth did not show promising yield performance. Among the accessions under study, performance of RO 5449 (37.1 g/t/t) and MT 4788 (34.3 g/t/t), MT 4796 (16.23 g/t/t), MT 4713 (13.04 g/t/t) and MT 6051 (11.75 g/t/t) were high compared to other accessions, while the check clone RRIM 600 was the highest yielder (45.5 g/t/t).The intrinsic drought tolerance potential of accession MT 4788 evidenced from the physiological evaluation under both field and laboratory screening is of significance here. It has been noticed in recent years that cold winters in North East India are followed by periods of high light intensity , which could lead to a further stress on the plants. The intrinsic capacity of accession MT 4788 to maintain high PSII activity under drought and light stress could have enabled good survival and yield under the cold climate of Agartala. There are several reports on the mechanisms of drought tolerance and cold tolerance being similar in many crop species. Since the same set of accessions could not be evaluated simultaneously for drought and cold tolerance, it may not be possible to draw conclusions from the present results.

Table 20: Range of variation in girth and yield among the wild germplasm accessions

Traits	Wild Genotypes Range	RRIM 600 (Check Clone)	General Mean
Girth at opening (cm)	39.4 (RO 5432) to 69.5 (AC 5502)	60.9	53.5
Forking height (m) 9^{th} yr.	2.04 (MT 6046) to 2.92 (MT 4713)	2.3	2.3
Bole volume (mt^3) 3 yrs after tapping	0.02 (RO 5853) to 1.0 (MT 4713)	0.5	0.34
Girth 3 yrs after tapping (cm)	44.1 (RO 5432) to 79.6 (AC 5502)	66.1	61.8
Yield (g/t/t) (Average of 3 years)	1.1 (RO 5055) to 37.2 (RO 5449)	45.5	7.3

The yield performance of different genotypes of the wild Brazilian germplasm from all three provenance of Mato Grosso, Rondonia and Acre showed that the genotypes from Mato Grosso provenance were better than those from Rondonia and Acre as also reported earlier by Abraham *et al.*, 1992 and Varghese and Abraham 1999 , from the traditional rubber growing region of India. When considering the growth performance, it was observed that Acre performed better than the accessions from Mato Grosso and Rondonia but considering limited number of Acre accessions, growth performance of Mato Grosso and Rondonia accessions were considered as superior (Table 21).

Table 21: Mean girth and yield of the accessions from different provenances

Source	No. of Accns.	Mean yield (g/t/t)	Mean girth at opening(cm)	Mean girth after 3 yrs of tapping (cm)
MatoGrosso	48	7.9	53.3	61.2
Rondonia	34	6.5	53.4	62.1
Acre	05	6.9	56.4	65.7
RRIM 600		45.5	60.9	66.1

7.2.3. Evaluations in the Northern part of West Bengal

The performance of 21 genotypes including a standard check clone in the climatic condition of North Bengal was studied to conserve selected germplasm for this region. The evaluation trial was laid out in 1998 at the Regional Experiment Station of the RRII in Nagrakata, situated in the foothills of the Himalayas in the Northern part of West Bengal where also, extremely cold winters limit growth and yield of *Hevea*.

Girth of the accessions ranged from 35.6 to 71.9 cm (Table 22). In MT 2229, MT 44, RO 2629, 2635, 2890, 3172, 5348, 5430 and 5557 girth was significantly higher than the check clone RRII 105. Accession RO 5363 showed the highest yield followed by AC 1950. Yield of RO 5363 was on par with the check clone RRII 105.

Table 22: Growth and yield in wild germplasm accessions at Nagrakata

Accessions	Girth at 12^{th} year of growth (cm)	Yield over four years (gm/tree/tap)	Accessions	Girth at 12^{th} year of growth (cm)	Yield over four years (gm/tree/tap)
AC1950	59.1	25.42	RO2629	65.2**	6.82
AC607	35.6	3.96	RO2635	66.7**	2.97

AC619	58.5	3.29	RO2890	71.9**	6.14
AC623	45.0	1.72	RO3172	70.9**	2.52
AC68	60.2	4.83	RO5329	55.7	13.70
AC763	53.7	19.03	RO5348	67.7**	4.21
MT196	60.6	19.68	RO5363	61.1	52.22
MT2229	64.7*	10.04	RO5408	55.0	5.44
MT2594	51.2	6.54	RO5430	64.1*	11.75
MT44	65.5**	8.35	RO5557	67.7**	5.96
			RO6139	51.8	4.78
RRII105	58.3	45.45			
			CD (0.05)	5.55	8.63

* Significant at 5% level. ** Significant at 1% level

Another set of 64 wild accessions were evaluated in Nagrakata for cold tolerance. In the seventh year of growth, girth of the accessions was recorded in the pre and post winter period. High girth was observed in MT 5105 (53.03 cm), RO 3204 (52.22 cm), AC 3353 (52.06 cm) and RO 2976 (50.09 cm) compared to SCATC 93/114 (46.49 cm) and RRIM 600 (48.44 cm) in Trial1. In Trial 2, RO 2727 recorded the highest girth of 53.19 cm followed by MT 915 (53.03 cm) and MT 900 (51.15 cm), while the controls RRIM 600 and Haiken 1 recorded 46.83 cm and 50.02 cm respectively.

Response in growth to cold stress was assessed using the girth increment values over the stress period in the two trials (Rao *et al.*, 2008). In Trial 1, AC 3300 had the highest girth increment of 1.69 cm.. The five accessions which showed highest girth values in their annual growth, had only medium rate of growth during the stress period as indicated by their girth increments. In Trial 2, maximum girth increment over the stress period was in RO 3043 (1.93 cm) Four accessions with highest annual girth recorded in the pre winter phase had high girth increment during the stress period.

8. Utilization of wild Amazonian *Hevea* germplasm

Apart from the relatively few direct selections for yield, utilization of the wild germplasm is possible only through hybridization of wild accessions containing the desirable allele, with elite cultivars. The domesticated Wickham and wild Amazonian populations being genetically divergent as already proved, W x A crosses have the potential to release transgressive segregants for more effective selection. 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 certain new genotypes with high yield as well as the desired wild allele. 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 vastly improve the chances of recovering a desirable new genotype in the next generation.

8.1. Heterosis breeding

A preliminary examination of the genealogy of parental clones involved in various crossing programmes of RRII has revealed that 45 common parents are involved, the most frequent ones being the two primary clones Tjir 1 and PB 86 followed by clones PB 24, Gl 1, PB 49, PB 56, PB 5/51, RRIC 52 and RRIM 600.The GAM cycle of mating followed by unidirectional selection for yield in the early years of *Hevea* breeding in India, coupled with vegetative propagation must have contributed to the loss of valuable genes in no small measure. The foregoing sections have detailed the efforts made by the multidisciplinary team of RRII to evaluate the 1981 IRRDB wild *Hevea* germplasm from various angles, so as to make available sufficient information on this valuable material for a proper choice of parents in future breeding programmes. Divergent crosses have been attempted in the last decade with the purpose of introgression of specific genes from these wild accessions to popular cultivated clones. Early results are encouraging.

8.1.1. Hybridization programmes in the traditional rubber growing region of Kerala

Superior wild accessions have been incorporated as parents in Wickham x Amazonian hybridization programs in various rubber research institutes. In India, W x A crosses were attempted in 1990, 1997, 2002, 2007 and 2009.

The performance of 26 hybrid clones (Table 23) resultant of the 1990 hybridization programme in which wild Amazonian germplasm was incorporated for the first time in *Hevea* breeding in India is shown in Figure1 in terms of girth and yield . Yield performance in a statistically laid out small scale trial over a period of seven years, yield in the peak and summer seasons, summer yield depression, girth at opening and in the seventh year of tapping, girth increment in the immature and mature phase, structural characters such as bark thickness and number of latex vessel rows at the time of opening the trees for tapping, important diseases and damage caused by wind were reported (Sankariammal *et al.*, 2010 a).

Seven W x A hybrid clones, out of which five belonged to the cross RRII 105 x RO 142, were better in yield over the control clone RRII 105. Mean yield ranged from 13.01 to 66.46g/t/t. The hybrid clone 90/10 recorded the highest yield followed by 90/34. Mean girth at opening of the hybrid clones ranged from 42.28 cm to 66.17 cm. The highest girth was recorded by clone 90/29. Girth in the 7th year of tapping ranged from 62.21 to 88.33cm, the highest was recorded in 90/29. Girth increment in the immature phase of hybrids ranged from 5.29 to 8.27 cm and in the mature phase it ranged from 1.91 to 4.81 cm. Twenty two clones attained average girth above 50 cm at opening. Bark thickness and latex vessel rows were high in clone 90/25. Incidence of major diseases and wind damage in general were low.

Divergent crosses are expected to result in heterotic progenies and the genetic divergence between Wickham clones and the wild Amazonian germplasm has been proved in earlier studies. Standard heterosis and heterobeltiosis were worked out in respect of the hybrid clones from the W x A crosses in India. Standard heterosis for yield (Table 24) ranged from 2.15 to 68.86 per cent and for girth it ranged from 4.58 to 63.66 per cent. Estimates of heterobeltiosis for yield ranged from 3.14 to 98.85 per cent and for girth ranged from 1.33 to 32.93 per cent. Seven clones *viz.*, 90/10, 90/25, 90/29, 90/34, 90/241, 90/170 and 90/271, which showed better yield and other secondary attributes have been selected for the next phase of evaluation in participatory trials.

Table 23: The first batch of W x A hybrid clones evolved in India

Sl. No.	Clone	Parentage	Sl. No.	Clone	Parentage
1.	90/3	RRII 105 x RO 142	14.	90/341	RRIM 600 x RO 142
2.	90/4	RRII 105 x RO 142	15.	90/168	RRIM 600 x RO 87
3.	90/6	RRII 105 x RO 142	16.	90/170	RRIM 600 x RO 87
4.	90/10	RRII 105 x RO 142	17.	90/171	RRIM 600 x RO 87
5.	90/19	RRII 105 x RO 142	18.	90/174	RRIM 600 x RO 87
6.	90/21	RRII 105 x RO 142	19.	90/176	RRIM 600 x RO 87
7.	90/25	RRII 105 x RO 142	20.	90/177	RRIM 600 x RO 87
8.	90/29	RRII 105 x RO 142	21.	90/184	RRIM 600 x RO 87

9.	90/34	RRII 105 x RO 142	22.	90/185	RRIM 600 x RO 87
10.	90/241	RRII 105 x RO 142	23.	90/193	RRIM 600 x RO 87
11.	90/321	RRIM 600 x RO 142	24.	90/352	RRIM 600 x RO 87
12.	90/322	RRIM 600 x RO 142	25.	90/265	RRIM 600 x RO 87
13.	90/340	RRIM 600 x RO 142	26.	90/271	RRIM 600 x RO 87

The second W x A hybridization programme of 1997 employed two popular Wickham clones viz. RRII 105 and RRIM 600 as female parents and nine genotypes from wild germplasm as male parents (MT 999, MT 1021, MT 1027, MT 1014, MT 1005, AC 495, AC 498, AC 817 and RO 380) which showed superior performance in certain secondary attributes. Fourteen cross combinations were attempted, a total of 3500 flowers were pollinated, 200 fruits (5.7 per cent) obtained, and the seeds after germination were planted in a seedling nursery in August 1997. The fruit set success of the crosses ranged from 1.15 to 16.43 per cent .The progenies were first evaluated in a seedling nursery (Sankariammal *et al.*, 2010 b) and the 27 selections with promising test tap yield compared to the high yielding check were multiplied and planted in a clonal nursery (Sankariammal and Mydin, unpublished).

In the 2002 hybridization programme, the high yielding clones, RRII 105 and its heterotic progeny clones, RRII 414 and RRII 429 were used as female parents in crosses with six promising wild accessions viz., AC 2004, AC 2584, RO 1739, MT 1057, MT 2226 and MT 1021.The 37 hybrid seedlings have been evaluated in the nursery for yield, girth, d.r.c. and structural attributes (Chandrashekar and Gireesh, 2008). The 2007 and 2009 HP utilized drought tolerant wild accessions and high yielding domesticated clones like RRII 105 and RRII 414 with the objective of developing high yielding drought tolerant *Hevea* clones. The progenies are under evaluation.

Figure 1 : Girth vs yield in the W x A hybrids

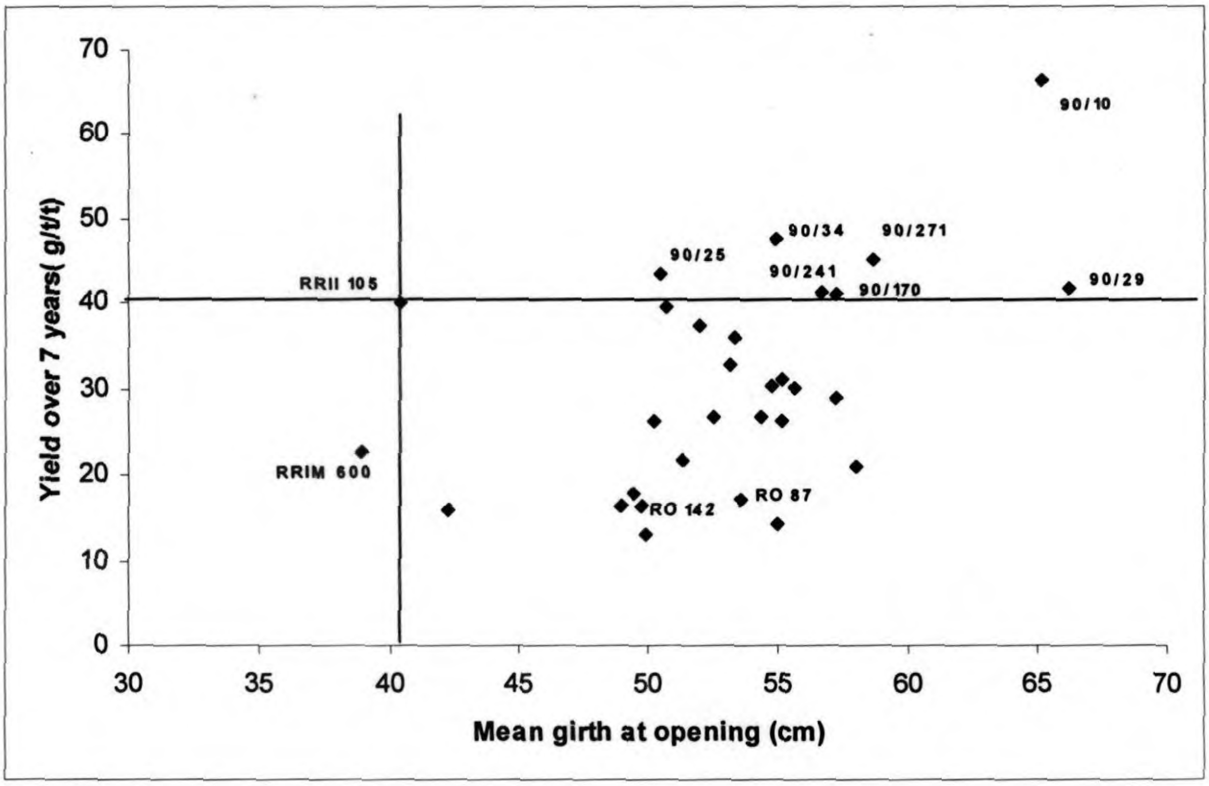

Table 24: Estimates of heterosis in W x A hybrids

		Standard heterosis(%)		Heterobeltiosis(%)	
Parentage	Clone	Girth at opening	Yield over five years	Girth at opening	Yield over 7 years
RRII 105 x RO 142	90/3	37.74	_	11.87	_
"	90/4	24.28	_	0.94	_
"	90/6	34.60	_	9.32	_
"	90/10	61.12	68.86	30.86	68.86
"	90/19	4.58	_	_	_
"	90/21	28.62	_	4.46	_
"	90/25	24.75	8.61	1.33	8.61
"	90/29	63.66	4.21	32.93	4.42
"	90/34	35.88	18.97	10.37	18.97
"	90/241	40.29	3.14	13.94	3.14

RRIM 600 x RO 142	90/321	43.66	_	16.67	_
"	90/322	36.45	_	10.83	37.57
"	90/340	25.53	_	1.95	75.28
"	90/341	22.24	_	_	-
RRIM 600 x RO 87	90/168	26.96	_	_	-
"	90/170	41.60	2.15	6.79	80.71
"	90/171	31.51	_	_	44.55
"	90/174	41.60	_	6.79	28.34
"	90/176	21.20	_	_	-
"	90/177	36.04	_	2.59	-
"	90/184	23.47	_	_	-
"	90/185	36.51	_	2.95	16.42
"	90/193	31.86	_	_	58.85
"	90/352	35.42	_	2.13	34.35
"	90/265	30.05	_	_	18.59
"	90/271	45.11	12.40	9.44	98.85

8.1.2. Hybridization to evolve cold tolerant clones in Tripura, N.E. India:

Selected potential wild accessions like AC 5502, MT 4788, MT 5946 and RO 5446 were utilized in the hybridization programme during 2006. Successful hybrids (RRIM 600 X RO 5449, RRIM 600 X MT 5946, RRIM 600 X MT 4788, GT 1 X MT 4788, GT 1 X AC 5502, GT 1 X RO 5449) were test tapped during October 2009 (with and without stimulation). Four seedlings are found promising (Table 25).

Table 25 : Juvenile yield of promising W x A hybrid seedlings

Cross combination	Test tap yield (g/plant/10 taps)		Girth (cm)
	Without stimulation	With Stimulation	
GT 1 X MT 4788	8.77	9.04	11.2

GT 1 x AC 5502	3.52	7.82	10.08
RRIM 600 X MT 4788	5.54	18.76	11.8
RRIM 600 X RO 5449	6.35	7.04	10.0

8.2. Polycross breeding

Apart from the laborious hand pollination programmes, an open pollination garden comprising 11 and 21 selected Wickham and Amazonian clones respectively, has been planted in 2005, with the objective of obtaining W x A cross seeds every year. Such seeds will have to be subjected to rigorous screening for desirable natural recombinants. More seed gardens will be planted as and when desirable wild accessions are identified. Eight accessions with promising performance for yield and timber potential have been planted during 2007 in a breeding garden comprising Wickham and Amazonian clones established at Rubber Research Institute of India. The RRII envisages the establishment of an arboretum to facilitate natural crossing among selected promising wild germplasm accessions as reservoirs of natural crossed seeds to obtain large base populations for selection and development of location specific clones.

9. Conclusion

India now maintains 4548 wild *Hevea* germplasm accessions from Acre, Rondonia and Mato Grosso provenances in *ex situ* conservation nurseries. Characterisation and evaluation of these valuable accessions have been progressing in a phased manner since the mid 1980s. As expected, wide variability in morphological, anatomical, physiological and biochemical attributes contributing to rubber yield, timber yield and quality, growth and secondary characters has been evident from evaluations done in phases by a multidisciplinary team of scientists of the RRII. Being a potential repository of genes that could impart tolerance/ resistance to biotic and abiotic stresses that the rubber tree is exposed to in the traditional and non- traditional regions of rubber cultivation in India, the RRII has been relentlessly pursuing measures for proper conservation , evaluation and above all, utilization of this precious germplasm for systematic and planned genetic improvement programmes. The current strategy for handling this valuable germplasm in India is outlined in Figure 2. Early results from the studies done at RRII have been promising. The W x A hybridization programmes effected since 1990 have resulted in some heterotic hybrids that have surpassed the popular high yielding clones in rubber and timber yield. Though few in number, there are also indications of direct selections for yield from this wild germplasm. The promising accessions, along with the heterotic hybrids and present day Wickham clones, could, over the next one or two generations unleash tremendous genetic variability which would be a boon to any *Hevea* breeder in the pursuit of bridging the gap between theoretical and realized yields in *Hevea brasiliensis* and expanding rubber cultivation to newer geographical locations.

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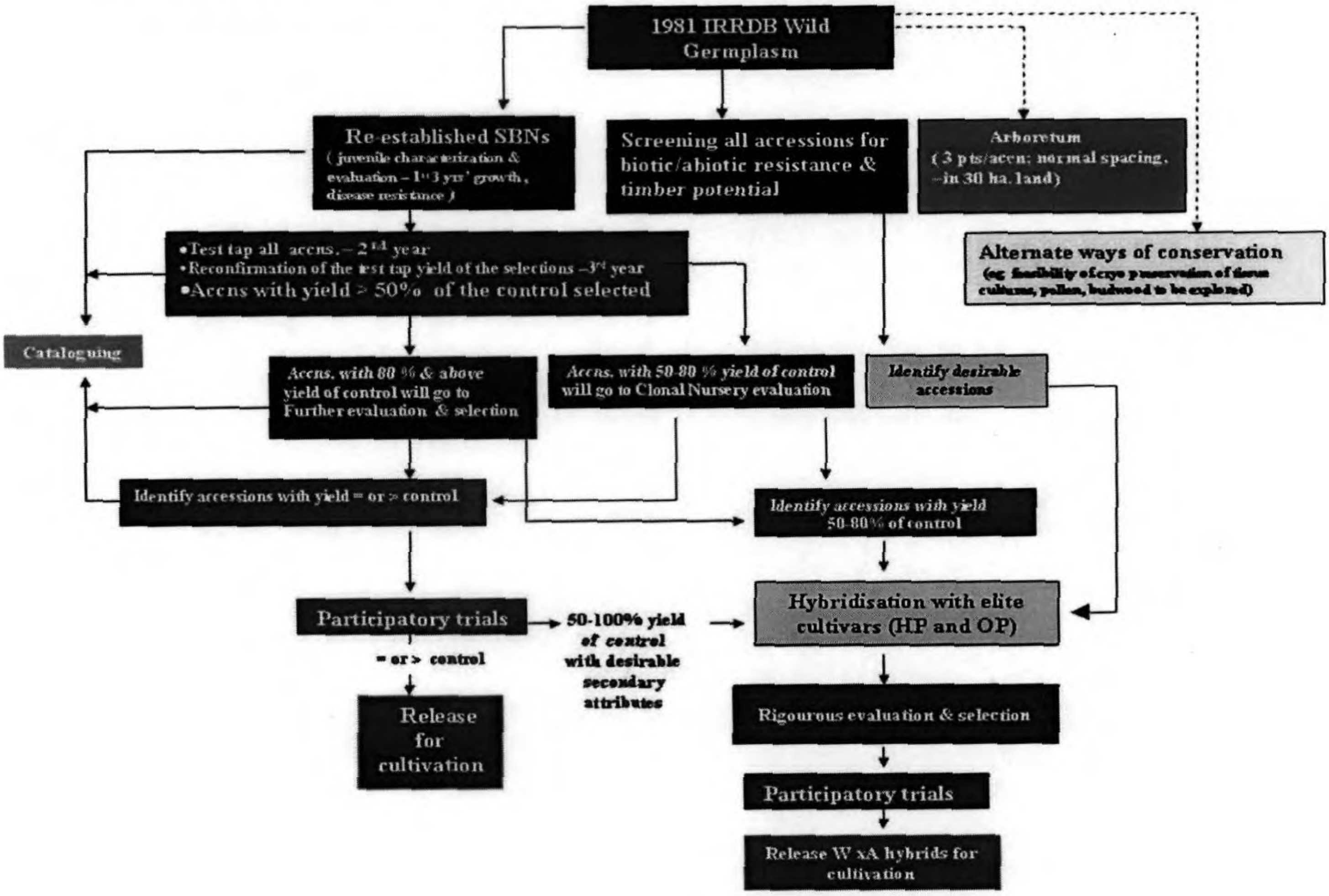
Figure 2: Strategy of conservation and utilization of the 1981 IRRDB wild *Hevea* germplasm collection



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