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NATURAL RUBBER RESEARCH

Important Information

The Rubber Research Institute of India, Rubber Board (under the Ministry of Commerce, Government of India) launched a scientific journal, *Indian Journal of Natural Rubber Research* during 1988 which was later renamed *Natural Rubber Research* in 2004. The scope of the journal was always limited to articles related to research on the biological and technological aspects of natural rubber.

The journal is celebrating its silver jubilee during 2012 and the first issue of volume 25 will be published in June 2012. Like any other scientific journal, our journal also experienced ups and downs in the last nearly quarter of a century of its existence, and the limited scope of the journal did not make things any easier.

Today, the journal is very much back on track; thanks to the committed work by its Editorial Board and the large number of articles that the journal is receiving. The first issue of 2011 is already out and the second issue is scheduled to be out in December 2011. Articles for the June 2012 issue are nearing finalization and articles for the December 2012 issue are under peer review.

The recent decades witnessed unprecedented developments in science and technology and the rubber sector, both the NR and the synthetic rubber sectors also reflected these developments. One significant lesson that we have learned over the years is that unless research on synthetic rubbers is also taken onboard complimenting with research on NR, the full impact of research on NR can not be realized in the rubber-goods manufacturing industry which is increasingly using NR:SR blends. While there is considerable scope for research on NR, this alone would not make the desired impact in the industry.

The Editorial Board of *Natural Rubber Research* has taken the following decisions that will be implemented from the silver jubilee volume onwards.

1. From 2012 onwards, the scope of the journal will include articles on synthetic rubbers in addition to natural rubber.

2. Theoretical /concept papers that have direct or indirect implications for research in natural rubber and synthetic rubbers will be included in the journal.

3. In addition to review articles, opinion papers and general articles of interest to rubber research or general interest to our readers will be published in the journal.

4. From 2012, journal subscription will be for a minimum period of three years (instead of the present one year) to save paperwork and the subscription rates will be Rs 3000/- (within India) and US$ 300/- (outside India) for the three years. A single issue of the journal will cost Rs 600/- (within India) and US$ 60/- (outside India). You can also subscribe the journal for a period of five years in which case the rates will be Rs. 4500/- (within India) and US$ 450/- (outside India). A subscription form is enclosed for your use.

5. Now that articles on synthetic rubbers also will be included in the journal from 2012, we propose to give a new name to the journal to reflect the change. We would be most happy to welcome your suggestion for a new name for the journal and also any other suggestions that will improve the quality of the journal. You may email your suggestions to james@rubberboard.org.in or send them by post to the following address.

RRII
01 November 2011

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Conservation of wild *Hevea* germplasm- Genetic diversity and selection of drought tolerant accessions

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ABSTRACT

India received 4548 wild germplasm accessions of Hevea brasiliensis as a result of the expedition conducted by International Rubber Research and Development Board in 1981 in the Amazon forests of Brazil, mainly concentrating in three states, namely Acre (AC), Rondonia (RO) and Mato Grosso (MT). This wild germplasm collection is conserved at Rubber Research Institute of India (RRII) and is now in various stages of evaluation. Wide variability observed in this large collection indicates that this collection has a broad genetic base, potentially important in broadening the existing narrow genetic base of cultivated rubber. Being a likely repository of genes conferring tolerance to various biotic and abiotic stresses, this wild collection is useful in developing Hevea clones tolerant to stresses, so that rubber cultivation can be extended to non traditional rubber growing areas experiencing adverse climatic conditions. As soil and atmospheric drought is a serious issue now-a-days limiting rubber cultivation, developing drought tolerant clones is very important. The potential use of wild Amazonian collection towards the development of drought tolerant Hevea clones is described in this paper, where accessions having drought tolerance potential are identified by conducting screening of this wild gene pool using various drought related morphological, physiological, structural and biochemical parameters related to drought tolerance.

INTRODUCTION

Present day rubber cultivation faces various climatic constraints such as prolonged soil moisture stress and high ambient day temperatures in the traditional as well as non-traditional rubber growing areas (Sethuraj *et al.*, 1989). Soil and atmospheric drought and high temperature are major environmental factors limiting growth and yield in *Hevea* which necessitates the development of drought tolerant clones suitable for such areas. A wide genetic variability within the base material is the primary requirement in breeding programmes aimed at selection for any specific trait. However, the narrow genetic base of the cultivated *Hevea* species developed from a minuscule of a genetic stock introduced by Sir Henry Wickham and the unidirectional selection for yield over the years in the cultivated clones limit the availability of wide genetic variability. Several investigations on drought resistance in crop plants have led to the observation that wild relatives of cultivated species are potential source of drought tolerance (Shimshi *et al.*, 1982). The wild accessions of *Hevea* collected through an expedition organized by the International Rubber Research and Development Board (IRRDB) in 1981 into the primary center of origin of the crop, the Amazon forests, is a valuable reservoir of genes conferring tolerance to various biotic and abiotic stresses (Varghese *et al.*, 2002). The exploration covered a wide range of agro climatic areas in the three Brazilian states of Acre (AC), Rondonia (RO) and Mato Grosso (MT).

Tolerance to drought is a highly complex polygenic character involving various morphological, anatomical, physiological, and biochemical traits. Moreover, drought often interacts with other stresses particularly temperature extremes, high light intensity and with biotic stress, making development of drought tolerant clones much more complex. Crop plants adapt to stress conditions by the intervention of several inductive morphological, anatomical, physiological and biochemical mechanisms more or less specific to species (Hanson, 1980; Kramer, 1983). Hence plant responses to water deficit have to be analyzed systematically by identifying traits that relate to drought tolerance. However, due to the specificity of time of sampling and elaborate protocols in such studies, it is not practically possible to study all these parameters simultaneously. Hence separate studies were conducted using different sets of this Amazonian gene pool, where a set of parameters related to drought tolerance were used for identifying potential accessions. The salient results obtained from these studies are discussed in this paper.

METHODOLOGY, RESULTS AND DISCUSSION

Accessions identified based on morphological parameters related to drought tolerance

There are various reports on the effect of drought on growth and yield of rubber. De Conceicao *et al.* (1986) noticed a reduction in growth and assimilate partitioning when *Hevea* clones were subjected to water deficit. In an analysis of growth and drought tolerance in rubber during the immature phase in a dry subhumid climate, Chandrashekar *et al.* (1998) noticed that a large portion of growth has occurred in the wet season while during the dry season the growth rates of the clones declined substantially. Most of the clones showed the same trend in girthing pattern also. Various other reports also suggest the changes occurring to plant stem, leaf and dry matter partitioning as a result of water stress. Hence a study was undertaken to evaluate the usefulness of some of the morphological traits for screening of germplasm for drought tolerance and to assess the genetic variability among the selected wild *Hevea* accessions for drought related parameters in an early growth phase (Mercy, *et al.*, 2006). Ten accessions were selected based on preliminary studies conducted among 450 accessions for growth parameters during summer months. In the field, 18 months old plants were used for assessing the genotypic difference for growth and vigour. The parameters recorded were scion height (cm), basal diameter of scion (mm), number of leaves, number of leaf flushes, interflush distance (cm), single leaflet area (cm^2) and Specific Leaf Weight

Table 1. Morphological parameters of selected accessions

Genotypes	Plant height (cm)	Basal diameter (mm)	No. of flushes	No. of leaves	Inter flush distance (cm)	Single leaflet area (cm^2)	SLW ($g\ cm^{-2}$)
AC 1044	102.79 ^{c}	11.14 ^{ab}	2.08 ^{cd}	49 ^{de}	28.05 ^{ab}	84.42 ^{a}	0.005 ^{a}
MT 55	147.06 ^{a}	13.23 ^{ab}	3.06 ^{abc}	90.89 ^{abc}	28.77 ^{ab}	87.05 ^{a}	0.006 ^{a}
AC 446	115.77 ^{abc}	13.49 ^{ab}	2.83 ^{abcd}	48.47 ^{de}	35.26 ^{ab}	86.84 ^{a}	0.005 ^{a}
RRIM 600	104.95 ^{c}	11.61 ^{ab}	2.28 ^{bcd}	51.44 ^{de}	22.67 ^{b}	58.82 ^{b}	0.006 ^{a}
Tjir 1	104.9 ^{c}	11.89 ^{ab}	2.61 ^{bcd}	70.92 ^{abcd}	22.18 ^{b}	59.30 ^{b}	0.007 ^{a}
MT 41	144.53 ^{ab}	12.88 ^{ab}	3.81 ^{a}	99.72 ^{a}	24.07 ^{b}	83.08 ^{a}	0.006 ^{a}
MT 76	144.06 ^{ab}	12.02 ^{ab}	3 ^{abc}	77.22 ^{abcd}	28.59 ^{ab}	60.85 ^{b}	0.006 ^{a}
MT 66	99.75 ^{c}	13.63 ^{ab}	2.69 ^{bcd}	48.25 ^{de}	19.57 ^{b}	81.56 ^{a}	0.007 ^{a}
MT 938	130.67 ^{abc}	11.5 ^{ab}	2.17 ^{cd}	60.33 ^{bcde}	43.92 ^{a}	84.8 ^{a}	0.006 ^{a}
AC 650	123.68 ^{abc}	15.79 ^{a}	3.36 ^{ab}	95.25 ^{ab}	32.51 ^{ab}	83.22 ^{a}	0.007 ^{a}
AC 652	99.22 ^{c}	10.14 ^{b}	1.78 ^{d}	33.45	21.72 ^{b}	80.44 ^{a}	0.006 ^{a}
RRII 105	108.31 ^{bc}	11.66 ^{ab}	2.25 ^{bcd}	50.08 ^{de}	20.15 ^{b}	61.52 ^{b}	0.007 ^{a}
AC 728	112.29 ^{abc}	13.1 ^{ab}	2.33 ^{bcd}	55.44 ^{cde}	24.47 ^{b}	90.78 ^{a}	0.005 ^{a}
G mean	118.31	12.47	2.64	63.88	27.07	76.784	0.006

Any two means having a common letter are not significantly different

(SLW). These characters give an indication of the general vigor of the genotype and hence studying these characters at the juvenile stage itself hold some importance.

The significant genotypic difference observed in almost all the morphological characters studied among the wild accessions (Table 1) indicated the wide genetic base of this material, which is highly useful for crop improvement. Plant height, which is an indication of rate of growth, among the accessions, varied from 99.22 to 147.06 cm. The tallest genotype was MT55, whereas the shortest was AC 652. In general, the wild accessions were taller than the Wickham clones. Pollinere (1996) reported that among the morphological components of yield, girth is considered as the most important parameter of growth and vigour in rubber. Hence, recording of basal diameter in the juvenile stage is important to assess the general vigour of the wild accession. Basal diameter was the highest in AC 650 (15.79 mm) followed by MT 66 and lowest in AC 652 (10.14 mm) with a mean of 12.47mm. The plant height and basal diameter in the juvenile phase give an indication of the general vigour of the plant and reports suggest that there will be a reduction in both as a result of water stress (Powell, 1976). Rate of production of leaves is another indicator of the vigour of the plants in the active growth phase. MT 41 recorded the highest number of leaves followed by AC 650, whereas the lowest number of leaves was present in AC 652 followed by MT 66. Number of flushes among the wild genotypes also exhibited significant genotypic difference. The maximum number of leaves and leaf flushes in the wild accession MT 41 along with comparatively high basal diameter and plant height indicate the vigorous growth habit of this accession. Such accessions are highly suitable for drought prone area. Leaf area is another important character to ascertain the photosynthetic capacity of the plants. The total leaf area is sometimes referred to as its photosynthetic potential (Ticha, 1985). In the improvement of plantation crops like rubber, effort has to be taken to bring light interception to 100% but without making the lower leaves parasitic. A plant having large leaves at the top could intercept almost all the light but would

Table 2. Morphological characterization based on drought sensitive parameters

Accession	Leaf size	Leaf surface	Wax coat	Yellowing (%)	Vigour	Senescence (%)
MT1631	Narrow	Flat	Nil	37.5	Low	100
MT 1710	Narrow	Curved	Nil	10	Medium	80
AC 462	Narrow	Flat	Nil	68.33	Medium	100
MT 3714	Narrow	Curved	Nil	100	Medium	84
AC 441	Narrow	Flat	Nil	100	Medium	100
MT 81	Broad	Flat	Nil	13.75	Medium	8.33
MT 200	Broad	Flat	Nil	61.25	High	45
RO 217	Narrow	Flat	Nil	40.0	High	34
RO 1520	Broad	Flat	Nil	88	Low	86
MT 1623	Narrow	Curved	Nil	7.5	High	0
MT 1681	Narrow	Curved	Nil	12.5	High	7.0
MT 52	Narrow	Flat	Nil	50.0	Low	54
MT 4242	Narrow	Flat	Nil	42.0	Medium	42
MT 60	Broad	Flat	Nil	76.25	Medium	80
MT 48	Broad	Flat	Nil	63.0	Medium	68
MT 1584	Broad	Flat	Nil	42.0	Medium	50
RRII 208	Broad	Flat	Nil	43.0	Medium	52
RRIM 600	Narrow/ small	Flat	Nil	48.0	High	47
Tjir 1	Narrow/ small	Flat	Nil	61.25	Medium	55
RRII 105	Narrow/ small	Flat	Yes	100.0	Medium	100

Table 3. The chlorophyll fluorescence parameters and percentage inhibitions in top ranking accessions.

Sl. No.	Accession	% inhibition in Fv/Fm	% inhibition in Q. Y.	%inhibition in Fv'/Fm'
Top ranking germplasm accessions				
1	MT 5100	6.0	4.3	4.2
2	MT 5078	12.6	4.5	7.6
3	MT 4788	10.0	8.8	10.7
4	MT 4856	14.0	10.0	20.0
Bottom ranking germplasm accessions				
1	MT 4694	54.0	37.0	47.0
2	RO 4615	30.0	30.7	24.5

result in so much shading that the efficiency of lower leaves would be reduced. Therefore selection for smaller leaves may provide a better plant canopy for higher photosynthetic efficiency. It has been reported that a small leaf size and greater leaf thickness are correlated to higher photosynthetic rate (Swaminathan, 1977). Single leaflet area of the accessions in the present study exhibited significant genotypic difference. Wild accessions in general recorded higher leaf area than the cultivated clones. The highest leaflet area recorded was 90.78 cm^2 in the accession AC 728 and the lowest was in MT 76 with a mean of 76.784 cm^2, indicating the genetic variation present among these accessions for the available transpiration area, which in turn helps the plants to adapt to periods of drought by reducing the transpiration loss. Genetic variation in leaf expansion rate among cocoa accessions under water stress has been reported by Balasimha (1987) and Joly and Hahn, 1989 suggested that the reduction in leaf area in cocoa plants help them to adapt to periods of drought. Chandrashekar *et al.*, (1990) noticed partial defoliation and leaf margin drying in *Hevea* clones during the summer periods in the non-traditional rubber growing area of North Konkan region as a part of reducing the total leaf area. All these reports suggest the importance of reduced leaf area under water stress condition. In this study, accessions like MT 76 and MT 41 with reduced leaf area are suitable for a water stressed condition. The mean inter flush distance recorded was 27.07cm with a range of 19.57 (MT 66) - 43.92 cm (MT 938). The inter flush distance indicates the transportation distance and hence a low inter flush distance is a positive sign where the translocation as well as partitioning of photosynthates will

Table 4. Clonal variation in leaf chlorophyll content after drought treatment (C- control, S- drought stressed)

Clones / Accessions	Chlorophyll	content (mg/g/fw)	% Reduction
RRII 105	C	3.67 ± 0.09	
	S	2.78 ± 0.09	24.5
RRIM 600	C	3.12 ± 0.16	
	S	2.86 ± 0.03	8.8
MT 4788	C	2.97 ± 0.11	
	S	2.40 ± 0.13	19.1
MT 5100	C	3.31 ± 0.16	
	S	2.95 ± 0.12	10.8
MT 4694	C	3.63 ± 0.25	
	S	2.85 ± 0.07	21.4

Table 5. Clonal variation in mid-day leaf water potential after drought treatment (C- control, S- drought stressed)

Clones/ Accessions		Mid-day leaf water Potential (-MPa)	% Reduction
RRII 105	C	2.4 ± 0.86	
	S	3.0 ± 0.40	25.2
RRIM	C	2.3 ± 0.58	
	S	2.7 ± 1.10	15.4
MT 4788	C	2.3 ± 0.53	
	S	2.9 ± 0.93	26.3
MT 5100	C	2.4 ± 0.31	
	S	2.8 ± 0.74	17.0
MT 4694	C	2.4 ± 1.03	
	S	2.9 ± 0.92	22.4

Table 6. Clonal variation in PS II quantum yield due to drought treatment (C- control, S- drought stressed)

Clones/ Accessions		PS II quantum yield	% Reduction
RRII 105	C	0.594 ± 0.01	
	S	0.349 ± 0.03	41.2
RRIM 600	C	0.533 ± 0.03	
	S	0.447 ± 0.01	17.0
MT 4788	C	0.486 ± 0.01	
	S	0.404 ± 0.01	11.0
MT 5100	C	0.569 ± 0.02	
	S	0.443 ± 0.03	22.5
MT 4694	C	0.582 ± 0.01	
	S	0.393 ± 0.04	32.5

Table 7. Percent leaf yellowing and senescence (ranges) of top, middle and bottom ranking accessions selected from Source Bush Nurseries (SBN) by empirical scoring

Germplasm Nursery	Yellowing (%)			Senescence (%)		
	Top	Middle	Bottom	Top	Middle	Bottom
SBN 2003	0 -5	14 -15	25 -40	0 -1	10 -15	20 -40
SBN 2004	0 -15	40 -45	60 -85	0 -1	15 -20	80 -90
SBN 2005	0 -10	30 -35	50 -80	5 -30	49- 50	75 -90
SBN 2006	10-14	39 -40	80- 90	10 -35	50 -55	90 -95
SBN 2007	0 -15	40 -50	50 -100	10 -30	49- 50	80 -100
SBN 2008	5 -15	40 -50	70-90	10 -30	50 -60	70 -100

Table 8. No. of wild accessions selected from germplasm source bush nurseries for intrinsic tolerance to drought stress.

Germplsm nursery	Total no. of accessions	Drought tolerant accessions	Drought susceptible accessions
SBN 2003	588	70	11
SBN 2004	976	49	30
SBN 2005	700	16	33
SBN 2006	806	11	32
SBN 2007	500	12	12
SBN 2008	202	7	3
Total	3772	165	121

be more effective. In addition to that they can provide mutual shading also. So the low inter flush distance recorded in the accessions MT 41, MT 76, MT 66 and AC 652 can be of more advantageous under drought stress conditions. In cocoa accessions, Balasimha, (1987) observed that the drought tolerant ones were those with high SLW. So the accessions with high SLW are preferred under drought conditions and hence the comparatively higher SLW noticed in the wild accessions MT 55, MT 41, MT 76, MT 66, MT 938, AC 650 and AC 652 would be a valuable trait for selection in germplasm material. In this study majority of the accessions with higher SLW appeared to be of Mato Grosso provenance highlighting their superiority under drought condition.

A pot culture experiment (Mercy *et al.*, 2010) using 16 wild *Hevea* germplasm accessions was conducted by inducing water stress. After experiencing moisture stress, accession MT 1623 showed the maximum girth whereas the total foliage production was more in MT 200. An assessment on mortality rate among the 16 accessions indicated 100% survival of two accessions *viz;* MT 1623 and MT 1681 under water stress. Girth increment of accessions with less than 50% mortality was assessed over a summer period and was found that it was high for MT accessions, again showing their potentiality for growth in the drought condition. In a preliminary screening carried out in 100 wild accessions in the traditional region in Kerala based on various drought related morphological, anatomical, physiological and biochemical characters, Mercy (2001) has reported the superiority of Mato Grosso accessions. 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 also done (Table 2) for identifying the potential drought

Table 9. Variability for leaf structural characters of selected accessions of *Hevea brasiliensis*

Accessions	No. of stomata per mm^2	Leaf thickness (μm)	Midrib diameter (μm)	Palisade tissue thickness (μm)	Mesophyll tissue thickness (μm)	Palisade no. per unit length
AC 1044	450.98^{ab}	123.93^{abc}	438.90^{a}	54.63^{bcde}	104.27^{abcd}	34.26^{ab}
MT 55	389.98^{abcd}	105.77^{fg}	310.67^{g}	48.04^{efg}	86.35^{ef}	28.60^{de}
AC 446	276.69^{d}	117.90^{bcde}	371.00^{bcd}	47.73^{efg}	84.95^{f}	30.22^{bcd}
RRIM 600	356.67^{abcd}	123.77^{abc}	352.20^{defg}	51.36^{cdef}	105.79^{abc}	35.24^{a}
Tjir 1	446.62^{ab}	129.83^{a}	406.80^{ab}	64.65^{a}	114.52^{a}	24.29^{f}
MT 41	333.33^{bcd}	104.23^{g}	360.73^{cde}	44.14^{fg}	82.67^{f}	33.51^{abc}
MT 76	357.30^{abcd}	127.83^{a}	346.48^{defg}	59.37^{abc}	102.78^{abcd}	31.19^{abcd}
MT 66	481.48^{a}	109.43^{efg}	419.53^{a}	48.31^{efg}	88.50^{def}	34.59^{a}
MT 938	296.29^{cd}	121.50^{abcd}	311.90^{fg}	42.42^{g}	101.38^{abcde}	33.47^{abc}
AC 650	331.16^{bcd}	114.43^{cdef}	355.18^{def}	50.43^{defg}	95.65^{bcdef}	29.76^{cde}
AC 652	348.58^{abcd}	127.70^{a}	312.93^{fg}	62.31^{ab}	109.79^{ab}	33.40^{abc}
RRII 105	427.01^{abc}	127.27^{ab}	400.20^{abc}	58.08^{abcd}	107.26^{abc}	32.38^{abcd}
AC 728	394.34^{abcd}	113.73^{def}	318.67^{efg}	51.64^{cdef}	92.47^{cdef}	26.04^{ef}

Any two means having a common letter are not significantly different

Table 10. Potential accessions identified for various biochemical parameters

Accessions with high chlorophyll content	Accessions with low chlorophyll reduction	Accessions with high wax content
RO 4599	MT 5131	RO 5062
MT 4878	RO 5072	RO 4599
MT 5090	RO 4595	RO 4620
AC 4939	RO 5004	RO 4595
RO 5163	AC 4676	RO 5044
RO 5162	AC 4939	RO 5022
	MT 4740	MT 5141
	MT 5093	MT 4704
	MT 5098	MT 4740
	MT 5100	MT 4870
	MT 5156	MT 5093
	RO 5163	MT 5098
		MT 5100
		MT 5156

tolerant accessions. It was found that among the 16 wild accessions studied, the accessions MT 1681 and MT 1623 were the potential ones for further detailed field evaluation towards developing a drought tolerant *Hevea* clone as these two were having many characters for drought adaptation.

Accessions identified based on physiological parameters related to drought tolerance

While measuring afternoon Leaf Water Potential (LWP) of 16 wild accessions (Mercy, *et al.*, 2010) under moisture stress, it was found that some of the MT accessions had higher LWP than the check clones. A screening of 182 wild *Hevea* germplasm accessions for intrinsic drought tolerance traits was also done (Nair *et al.*, 2005). Leaf discs were punched (size, 2.0 cm diameter), washed and floated in Polyethylene Glycol Solution (60% solution of PEG- 6000, Merck India Ltd.) with a known osmotic potential of -38 bars. These samples were simultaneously exposed to 350 μmole

m^{-2} s^{-1} of fluorescent light for four hours at a constant temperature of 25^0 C. A similar set of samples was incubated in distilled water in dark (as control). For standardization of the technique polyethylene glycol at concentrations of 20% (-5.3 bars), 40% (-16 bars), 60% (-38 bars) and 80% (-57 bars) were used.

All samples were washed repeatedly in distilled water after incubation and kept in dark for 30 minutes dark adaptation. Leaf clips were used to avoid any outside light falling on the samples for accurate measurements. Chlorophyll fluorescence (dark Fv/ Fm) was measured using a Pulse Amplitude Modulated Fluorometer, PAM- 2000 (Walz, Germany). The samples were then exposed to an actinic light of 100 µmole m^{-2} s^{-1} for few seconds. The quantum yield of electron transport at photo system II (Q.Y.) and the efficiency of excitation energy capture by open PS II (Fv'/Fm') were measured. The efficiency of photosynthetic process was monitored in leaves using the chlorophyll fluorescence technique. Wide variation of reductions in maximum potential photochemical efficiency (dark Fv/Fm), the Quantum Yield of electron transport (Q.Y.) and the efficiency of excitation energy capture by open PS II reaction centers (Fv'/Fm') were noticed upon incubation of leaf tissues in PEG and exposure to light. Among the 182 accessions screened, four Mato Grasso (MT) accessions *viz.* MT 5100, MT 5078, MT 4788 and MT 4856 showed smaller reductions and consistently appeared in the top ten ranks for all three parameters studied. These four MT accessions showing intrinsic tolerance traits were selected as drought tolerant accessions. Similarly, two accessions *viz.* MT 4694 and RO 4615 were identified as the most susceptible ones as they were consistently present in the bottom ten ranks in all three parameters studied (Table 3).

The adverse effect of multiple environmental constraints were further evaluated (Nair *et al.*, 2011) in polybag grown plants of clones RRII 105, RRIM 600 and germplasm accessions *viz.* MT 5100, MT 4788, MT 4694 subjected to soil drought by withholding irrigation for three weeks. The reduction of leaf chlorophyll content and mid-day leaf water potential was more in drought susceptible accessions than the tolerant ones (Table 4&5). Under drought the PS II quantum yields declined more in drought susceptible clone RRII 105 (41%) than drought tolerant RRIM 600 (17%) and the germplasm accessions exhibited less damage than the drought susceptible clone RRII 105 (Table 6).

To identify drought tolerant accessions from wild germplasm, field scoring for visual drought tolerance traits was carried out in 3772 accessions (Nair *et al.*, 2011). Empirical field scoring of leaf yellowing and senescence ranged from 0 to 100% (Table 7). The scored values were ranked and genotypes exhibiting tolerance to drought stress *i.e.* plants showing low leaf yellowing and senescence (low score) were ranked top. Similarly, middle ranking and bottom ranking ones were selected. A total of 165 wild accessions with intrinsic tolerance to drought stress (top ranking ones) and 121 accessions susceptible to drought stress (bottom ranking ones) were grouped (Table 8).

The short listed accessions (top, middle and bottom ranking ones) were subjected to *in vitro* drought (PEG) and light stress and a wide variation in PS II photochemical efficiency was observed. The top ranking accessions that showed less yellowing and less senescence in field exhibited higher PS II activity, whereas, accessions showing more yellowing and more senescence exhibited higher reduction in PS II activity under *in-vitro* drought and light.

Accessions identified based on structural (leaf, bark, and stem) parameters related to drought tolerance

The role of number and size of stomata, amount of palisade tissue, thickness of mesophyll and spongy parenchyma, waxy cuticle etc. in leaf as anatomical markers towards drought tolerance has been reported by Rajagopal *et al.* (1990), Sam *et al.* (1996), Mei

Xiuying *et al.* (1998). Among the stem structural characters, internal phloem in some higher plants is related to physiological processes involved in drought resistance (Esau, 1974). Using these parameters, ten accessions selected based on preliminary studies conducted among 450 accessions were screened using polybag plants (Mercy *et al.*, 2004).

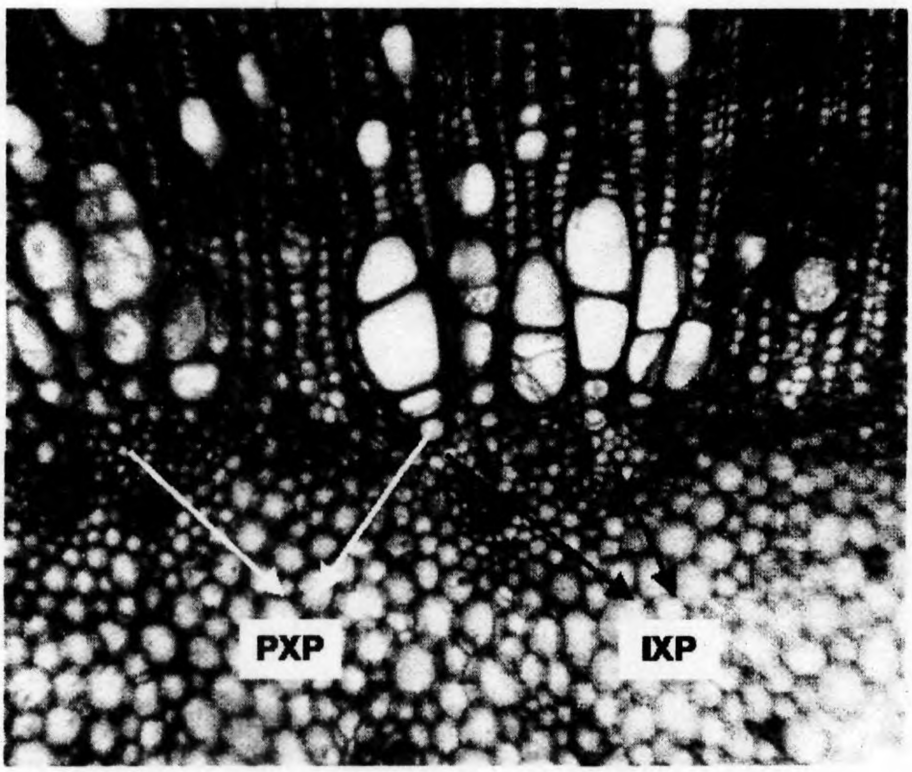
Physiologically mature leaves from the top whorl of non stressed plants were collected and leaf anatomical parameters such as thickness of palisade tissue (µm), thickness of mesophyll tissue (µm), mean number of cells in unit length of palisade layer, leaf lamina thickness (µm) and leaf vein (midrib) diameter (mm) were recorded from the cross section of the leaf. For studying stomatal density, epidermal peelings were taken by boiling the leaf bits in 60% Nitric acid with a pinch of potassium chlorate and the stomatal count of the lower epidermis was taken and expressed as number of stomata per square mm.

The number of stomata per mm^2 of leaf lamina (Table 9) varied from 276.99 to 481.48, with a mean stomatal density of 376.19. Accession AC 446 had the lowest number of stomata followed by MT 938 and the highest was in the accession AC 1044. In majority of the wild accessions the stomatal number per unit area of leaf lamina was lesser than the mean value. Stomatal pores, which are minute intercellular openings on the leaf surface, play an important role in the water balance system of the plant. Since higher number of stomata per plant leaf surface would increase the transpirational water loss for crops growing under rain fed condition, a low number of stomata per plant is found to be desirable (Jones, 1977) and hence a comparative less number of stomata present among the wild *Hevea* germplasm is advantageous.

Leaf thickness of the accessions studied varied from 104.23 to 129.83 µm and the mean leaf thickness was 119.03 µm. The highest leaf thickness was noticed in the wild accession MT 76 followed by AC 652. Pearcy (1998) suggested that both adaptive and genetic differences in the rate of photosynthesis per unit leaf area are associated with differences in leaf thickness. For midrib diameter, the range was 310.67 - 438.9 µm with a mean of 361.94 µm. The highest midrib diameter was recorded in the wild accession AC 1044 followed by MT 66 and the lowest was in MT 55 and in MT 938. Thickness of palisade tissue ranged from 42.42 to 64.65 µm, with a mean value of 52.55 µm. Among the wild accessions the maximum palisade tissue thickness was noticed in AC 652 followed by MT 76 whereas the lowest was in MT 938 and in MT 41. The mesophyll tissue thickness measured was in the range of 82.97 - 114.52 µm and the accession AC 652 recorded the highest mesophyll tissue thickness (109.79 µm) among the wild accessions, while the lowest was in MT 41. Palisade cell number per unit length recorded varied from 24.29 to 35.24 with a mean of 31.3. Among the wild accessions, the maximum palisade cell number was seen in MT 66 (34.59) followed by AC 1044 (34.26) and the lowest was in AC 728 (26.04).

Important leaf structural characters that take part directly or indirectly in the water regulation and gaseous exchange of the plant are thickness of leaf blade and midrib, thickness of palisade and mesophyll tissue, and number of cells in unit length of palisade layer. All these characters are associated with photosynthetic capacity of the plant. Midrib diameter is important for the translocation of photosynthates from sites of their production. Mesophyll tissue especially the palisade tissue is important for photosynthetic activity due to the presence of chloroplast in them. According to earlier reports, the wild accessions with greater values for leaf thickness, midrib diameter, palisade and mesophyll tissue thickness and palisade cell number per unit length of palisade tissue can be selected for drought situations.

Internal phloem as an indicator of breeding value for drought resistance in an interspecific hybrid of cassava has been



PXP – primary xylem points; IXP – intraxylary phloem

Fig.1. Transverse section of a twig showing primary xylem and intraxylary phloem in *Hevea brasiliensis*

reported by Graciano-Ribeiro *et al.*, 2009. Internal phloem is very efficient for conduction, and consequently, is of great advantage for desert plants, which are under shorter but more intense photosynthetic periods (Fahn 1990). Anatomical observations on primary xylem points and intra xylary phloem were studied among 16 wild accessions using twig samples of one year growth (Mercy, *et al.*, 2010). Transverse section of twigs was prepared at 60-80 µm thickness. The sections were observed under a bright field microscope and stained in Toluidene Blue O. Number of Primary Xylem Points (PXP) and number of Intraxylary Phloem Points (IXP) were quantified, and diameter of twigs were recorded and compared. Five sections per twig were observed for recording the characters.

In *Hevea*, Intra Xylary Phloem (IXP) appears in the peri central region of the stem as strands flanking the Primary Xylem Points(PXP) groups (Fig. 1). In *Hevea*, the occurrence of adaxial (medullary) phloem associated with protoxylem was noticed first, by Premakumari *et al* (1985). Premakumari and Panikkar (1988) and Premakumari (1992) further observed that PXP and IXP are highly significant clonal characters and there is a positive correlation between the number of intra xylary phloem points and rate of girth increment under tapping. According to Zamski and Tsivion, (1977) in tobacco, girdling activated the internal phloem to take on the functions of external phloem for transporting assimilates. The wild accessions showed wide variation with respect to PXP, IXP and diameter of twigs. The number of primary xylem points varied from 29.20 (MT 4242) to 104.50 (MT 1623). With respect to IXP, the accession MT 1623 recorded significantly superior values compared to the check clone RRIM 600, which is a proven drought tolerant clone. Four accessions viz., MT 1623, MT 200, MT 3714, and MT 48 showed better values than the general mean. Among the check clones, the highest IXP was recorded for RRIM 600 followed by RRII 208 whereas, in Tjir 1 which is a drought susceptible clone it was relatively low. Three accessions viz., MT 1623, MT 200 and MT 3714 recorded higher values for this trait as compared to RRIM 600, of which the former two accessions were significantly superior to RRII 208. The variability for IXP among the different accessions was higher than that of the variability for PXP, as indicated by the high CV for this trait. Diameter of twigs varied from 7.50 (MT 52) to 20.30 mm (MT 200). A relative comparison of the proportion of IXP per mm. diameter of twig also showed the superiority of the accession MT 1623.

Accessions identified based on biochemical parameters related to drought tolerance

Total chlorophyll content, chlorophyll degradation, epicuticular wax content and electrolyte leakage from cell membrane caused by membrane injury were used for screening a set of germplasm materials for drought tolerance (Mercy *et al.*, 2005). Physiologically mature leaves were collected for estimation from three plants of uniform growth and maturity in each accession. Total chlorophyll was estimated by the method of Ozerol and Titus (1965). Chlorophyll reduction percentage was worked out based

Table 11. Variation for cell membrane injury among the selected accessions

Accessions	% injury	Accessions	% injury
AC 4677	27.1	MT 5125	19.6
RO 5047	25.6	MT 4740	23.6
MT 4694	17.9	MT 5093	20.3
MT 4878	55.5	RO 4595	32.4
RO 5023	66.6	RO 4620	42.1
AC 4833	55.7	RRII 105	36.9
MT 5156	67.1	RRIM 600	18.0
Mean = 36.3	Variance Ratio= 34.02**		
SD = 5.35	** Significant at P = 0.01		

on pigment changes induced by heating (Dhopte and Livera, 1989). The wax content was determined by the gravimetric method of isolation (Silva Fernandes *et al.*, 1964). 12 potential accessions from 37 were selected and they were subjected to electrolyte leakage study by using modified procedure of Sullivan (1972) reported by Rajagopal *et al.* (1988) in *Hevea* where the accessions were subjected to a combination of water and heat stress.

The total chlorophyll content showed a wide range which varied from 2.4 to 4.6 mg cm^{-2}. The highest chlorophyll content of 4.6 mg cm^{-2} was recorded in two accessions MT 5090 and RO 5163, where as the lowest was observed in the accession MT 5098 (2.4 mg cm^{-2}). The variation among the accessions for total chlorophyll content clearly indicates the genetic variation among the wild germplasm accessions. As there is a strong correlation between the chlorophyll content and photosynthetic efficiency of the plant, the accessions with high chlorophyll content are the potential accessions suitable for growing in drought prone areas.

The reduction percentage varied from 2.7 to 26.8% and the lowest chlorophyll reduction was noticed in the wild accession MT 5156 (2.7%) followed by MT 5098 (3.2%) whereas the highest reduction was in the accession RO 5047 (26.8%). The decrease in chlorophyll content due to high temperature stress and water stress was due to loss of chloroplast membrane integrity under water deficit. Chlorophyll reduction percentage is negatively correlated with drought, and hence low chlorophyll reduction percentage observed in wild accessions MT 5156, MT 5098, MT 5131, AC 4939 and AC 4676 are highly appreciable.

The total wax content among the accessions showed a wide range which varied from 37.5 to 183.6 µg cm^{-2} with the highest in the accession RO 4595 (183.6 µg cm^{-2}) followed by RO 4620 (165.0 µg cm^{-2}) whereas the lowest was in the wild accession AC 4677 (37.5 µg cm^{-2}). ECW content increases due to stress and helps the plants to withstand drought. The role of ECW in the maintenance of water balance has been reported in various crops such as cocoa (Balasimha *et al.*, 1985), rubber (Gururaja Rao *et al.*, 1988) coconut (Rajagopal *et al.*, 1989) and in wild accessions of *Hevea* (Mercy, 2001). Higher

Table 12. Potential accessions with good juvenile yield

Accession	Test tap yield (g) of 10 tappings	Accession	Test tap yield (g) of 10 tappings
RO 1769	33.34	MT 62	11.06
RO 2976	21.67	RO 3626	11.05
AC 173	15.14	MT 1660	9.47
MT 915	14.72	RRII 105	21.99
MT 2229	11.09	RRIM 600	24.08
		CD	13.31

wax content helps in the adaptation of the plant to the drought conditions by reducing the stomatal conductance and transpiration rate. In addition to this, the presence of ECW on the leaf surface helps to reflect the excess solar radiation and there by maintaining the leaf temperature to a minimum level. Comparison of wax was found to vary with species, seasons and also with the intensity of light which will affect the cuticular transpiration as reported by Baker, (1974). In Table 10, the list of potential accessions selected based on the above mentioned parameters is given.

The extent of variability among the selected accessions, for Cellular Membrane Stability (CMS) as indicated by relative injury to cell varied from 18 to 67% with a mean of 38%. The accessions MT 4694, MT 5125 and MT 5093 showed highest tolerance to water and temperature stress and stability on par with RRIM 600 as indicated by low relative injury (Table 11). PolyEthylene-Glycols (PEG) of high molecular weight have long been used to stimulate drought stress in plants as a non-penetrating osmotic agent lowering the water potential in a way similar to soil drying (Larher *et al.*, 1993). The combined treatment of samples with PEG and heat is a suitable method for estimating the stress tolerance of *Hevea* to water and high temperature stresses (Nair *et al.*, 1995; Mercy, 2001). The significant genotypic differences for CMS indicate difference in their cellular sensitivity to desiccation stress and the accessions coming under the extreme group with low cellular injury indicate their drought tolerance potential.

Table 13. Accessions showing better performance for mean dry rubber yield

Accessions	Summer season yield (g/t/t)		Peak season yield (g/t/t)		Total yield (g/t/t)		% of the yield of RRII 105	
	4th yr.	5th yr.	4th yr.	5th yr.	4th yr.	5th yr.	4th yr.	5th yr.
AC 166	31.64	46.03	57.06	66.32	49.40	56.08	108.00	91.20
MT 1020	27.96	18.00	50.97	36.55	48.68	26.97	106.00	43.00
MT 179	20.37	14.37	49.78	32.61	45.35	23.57	99.00	38.00
RO 2908	25.96	16.48	56.99	56.08	44.45	43.78	97.00	71.00
AC 675	20.55	13.89	49.88	35.93	43.44	26.09	94.80	42.00
RO 2385	21.81	15.24	49.01	40.11	41.12	32.57	89.80	52.80
AC 655	20.04	20.45	38.67	40.69	39.44	30.80	86.00	50.00
AC 2004	21.32	23.44	47.34	46.50	39.44	34.86	86.00	56.70
MT 54	22.40	15.49	41.04	30.73	35.35	20.37	77.00	33.00
AC 670	18.95	28.29	27.77	52.85	26.99	40.78	59.00	66.00
RRII 105	23.44	41.18	44.51	82.73	45.79	62.27		
CD(5%)	9.10	10.91	19.44	19.74	13.23	14.78		

Accessions identified by screening in hot-spot region (at RRS, Dapchari, Maharashtra)

Regional Research Station (RRS), Dapchari located in Maharashtra state in India, is a drought prone region experiencing high temperature (exceeding 40°C in

April), high light intensity and very low soil moisture during the summer months. It has a rainfall pattern limited only to four months in a year, with an average annual rain fall of 7.5mm per day and an average of 90 rainy days in a year. Preliminary field screening of wild germplasm in this drought prone region was conducted to identify potential drought tolerant accessions. Two sets of 63 and 42 wild accessions were screened (Mercy *et al.*, 2009) for three years for assessing their growth and clonal response towards drought stress experiencing from February to May at RRS, Dapchari. From the set of 63 accessions, the potential accessions identified were MT 1668, MT 67, MT 1616, MT 1649, MT 1627 and MT 54. In general, accessions from the MT provenance showed superiority for their growth and survival in a drought prone area compared to the accessions from the other two provenances. Comparatively prolonged dry season prevailing in the state of Mato Grosso might have resulted in better adaptability to drought for these accessions. Accessions MT 179, RO 4184, MT 56 and MT 196 were the potential ones identified from the set of 42 accessions.

In another set of 130 accessions screened (Mercy *et al.*, 2012), fourteen potential accessions could be identified for further detailed field evaluation based on girth (vigor) and juvenile yield. The accession MT 1697 ranked first for vigor and the rate of leaf senescence in post-stress period was the lowest in the accession MT 4222. Accessions MT 1697, MT 4222, MT 1623 and RO 2889 showed superiority for more than one character. Accession RO 1769 gave juvenile yield higher than the check clones RRIM 600 and RRII 105 and the same for RO 2976 was also very promising (Table 12). The promising yield obtained from the wild accessions even in an adverse climatic condition highlight the merit of these accessions clearly indicating the genetic potential of this wild gene pool.

In another detailed evaluation going on at RRS, Dapchari (Mercy *et al.*, 2011), it was noticed that among the selected 19 potential wild accessions, accession MT 4788 recorded highest girth at the age of four years and it was more than that of the check clone RRII 430 (a drought tolerant check). In the traditional belt, this accession has recorded mature yield higher than the known high yielder RRII 105 (unpublished data) and hence the vigorous growth of this accession even at drought prone region is having great scope. After experiencing the three summer periods of 2008, 2009 and 2010, eight wild accessions out of 19 recorded higher girth than the proven drought tolerant clone RRIM 600 and there were 11 wild accessions with girth greater than RRII 208, another proven drought tolerant clone. This shows the differential growth response of wild accessions in a drought prone area resulted due to wide genetic base of this material.

Accessions identified based on mature yield (at RRS, Padiyoor, Kerala)

Even in traditional areas drought stress is common and this occur concomitant with high temperature and high light during summer. Regional Research Station located at Padiyoor of Northern Kerala is a place experiencing high temperature and high light during summer. Hence, field evaluation of wild *Hevea* germplasm conducted in this region is also having importance. An evaluation of a set of 80 accessions was conducted in this region to identify potential accessions for mature yield. Of the 80 wild accessions evaluated, 14 accessions had the summer and peak season yield statistically on par with that of RRII 105 in the 4th year (Table 13) and accession AC 166 was the highest yielder (Reghu *et al.*, 2012). RRII has started on-farm evaluation trials using this accession at five different estates. The 14 potential accessions identified for mature yield will be promising at drought prone region also as these accessions experienced similar drought situation though they were grown in the traditional belt.

CONCLUSION

Natural rubber cultivation in India faces adverse effects of drought and cold stresses especially in the non-traditional rubber growing areas (Jacob *et al.*, 1999). The genetically divergent Amazonian accessions are an excellent repository of various useful traits including stress tolerance. If we are able to tap the full potential of this wild gene pool, we are able to identify candidate genes from this collection, for developing location specific clones for non-traditional rubber growing areas.

REFERENCES

Baker E. A., 1974. The influence of environment on leaf wax development in *Brassica oleracea var. gemmifera*. *New Phytol.* **73**:955-966.

Balashimha D., 1987. Cocoa. In: 'Tree Crop Physiology, Eds.: Sethuraj M.R. and Raghavendra A.S.'. Elsevier, Amsterdam: 263-285.

Balasimha D., Subramanian N. and Subbaiah C. C., 1985. Leaf characteristics in cocoa (*Theobroma cacao L.)* accessions. *Café Cacao The.* 29: 95-98.

Chandrasekhar T.R., Jana M.K., Thomas J., Vijayakumar K.R. and Sethuraj M.R., 1990. Seasonal changes in physiological characteristics and yield in newly opened trees of *Hevea brasiliensis* in north Konkan. *Indian J. Nat. Rubb. Res.* 3(2): 88-97.

De Conceicao H., Oliva M.A. and Lopes N.F., 1986. Drought resistance in the rubber tree. II. Growth and assimilate partitioning of six clones subjected to a water deficit. *Pesquisa – Agropecuria - Brasileria* 21(2): 141-153.

Dhopte A. M. and Livera M. M., 1989. Chlorophyll stability index for drought tolerance in plants. Useful techniques for plant scientists. Publications for plant physiologists, Akola:102-103.

Esau K., 1974. O Caule: Estágio Primário de Crescimento. In: 'Anatomia das Plantas com Sementes (Ed: Blücher E.). Edgard Blücher Ltda., São Paulo: 160-185.

Graciano-Ribeiro D., Hashimoto D.Y.C., Nogueira L.C., Teodoro D., Miranda S.F. and Nassar N.M.A., 2009. Internal phloem in an interspecific hybrid of cassava, an indicator of breeding value for drought resistance. *Genetics and Molecular Research* 8 (3): 1139-1146.

Fahn A., 1990. Plant Anatomy, 4th Ed.: Pergamon, New York. p. 588.

Flores-Vindas E., 1999. Floema. In: 'La Planta: estructura y function'. Cartargo : 287-315.

Gururaj R.G., Devakumar A. S., Rajagopal R., AnnammaV., Vijaya kumar K. R. and Sethuraj M. R., 1988. Clonal variation in leaf cuticular waxes and reflectance: possible role in drought tolerance in *Hevea*. *Indian J. Nat. Rubber Res.* 1: 84-87.

Hanson A.D., 1980. Interpreting the metabolic responses of plants to water stress. *Hort. Sc.* 15: 623-629.

Jacob J., Annamalainathan K., Alam B., Sathik M.B.M., Thapaliyal A.P. and Devakumar A.S., 1999. Physiological constraints for cultivation of *Hevea brasiliensis* in certain unfavorable agroclimatic regions of India. *Indian J. Nat Rubber Res.* 12(1&2): 1-16.

Joly R.J. and Hahn D.T., 1989. Net CO2 assimilation of cocoa seedlings during periods of plant water deficit. *Photosyn. Res.* 21:151-159.

Jones H.G., 1977. Aspects of the water relations of spring wheat (*Triticum aestivum.* L) in response to induced drought. *J. Agric. Sc.* 88: 267-282.

Kramer P.J., 1983. Water deficits and plant growth. In 'Water Relations of Plants (Ed.: Kramer P.J.)'. Academic Press, New York: 342-389.

Larher H., Leport L., Petrivalsky M. and Chappart M., 1993. Effectors for the osmoinduced proline response in higher plants. *Plant Physiol. Biochem* 31 (6):911-922.

Mercy M. A., 2001. Genotypic evaluation and screening for drought tolerance in wild *Hevea* germplasm. Ph. D. Thesis, Kerala Agricultural University, Trichur, India. p.115.

Mercy M.A., Abraham S.T., George P.J., Potty S.N., Sethuraj M.R. and Saraswathi P., 1993. Preliminary observations of the 1981 IRRDB *Hevea* germplasm. II. Variability, dry matter and morphological characters. *Journal of Plantation Crops* 21 (Supplement): 268-274.

Mercy M.A., Babu L.C. and Varghese Y.A., 2004. Evaluation of wild *Hevea* germplasm for drought tolerance based on leaf and bark anatomical characteristics. *Journal of Plantation Crops* 32 (Supplement): 87- 94.

Mercy M.A., Jose G. and Varghese Y.A., 2005. Screening of wild germplasm of rubber (*Hevea brasiliensis*) for drought tolerance based on chlorophyll and wax content, chlorophyll degradation and membrane stability. Preprints of Papers, International Natural Rubber Conference, 2005, India: 135-144.

Mercy M.A., Babu L.C. and Varghese Y.A., 2006. Early evaluation of wild *Hevea* germplasm for drought tolerance based on growth and dry matter production. *Journal of Plantation Crops* 34: 631-638.

Mercy M. A., Meena Singh, Reghu C. P. and Varghese Y.A., 2010. Preliminary field screening of wild *Hevea* germplasm for tolerance to drought. *Natural Rubber Research* 23(1&2): 71-79.

Mercy M.A., Meenakumari T. and Varghese Y.A., 2010. Pot culture experiment on screening for tolerance to drought in a set of wild *Hevea* germplasm. *Indian Journal of Plant Genetic Resources* 23(1): 4-10.

Mei Xiuying, Jiang Zaimin, Gao Shaotang, Cui Hongan, Liu Chaobin and Cao Yumei, 1998. A study on the anatomical structures of the leaves of 14 *Juglans regia* and *Juglans sigillata* varieties (elite clones) and their drought resistance. *J. Northwest Forestry College* 13(1): 16-20.

Nair D.B., Dey S.K., Rajagopal R., Vijayakumar K.R. and Sethuraj M. R., 1995. Synergistic effect of heat and osmotic stress in causing membrane injury in *Hevea brasiliensis*. *Proc. IRRDB Symposium on Physiological and Molecular Aspects of the Breeding of Hevea brasiliensis*, 6-7 November, Penang, Malaysia: 177-181.

Nair D.B., Jacob J., Mercy M.A., Varghese Y.A. and Alam B., 2005. Screening of wild *Hevea* germplasm accessions for intrinsic drought tolerance traits. Preprints of papers, International Natural Rubber Conference, 2005, India: 62-67.

Nair D.B., Mercy M. A., Annamalainathan K., Krishnakumar R. and Jacob J., 2011. Physiological traits for identification of potential drought tolerant accessions from wild *Hevea* germplasm. *Natural Rubber Research* 24(1): 69-75.

Ozerol N. H. and Titus J.S., 1965. The determination of total chlorophyll in methanol extracts. Transactions of the Illinois State Academy of Sciences 58: 39.

Pearcy R.W., 1988. Acclimation to sun and shade. In 'Photosynthesis – A comprehensive Treatise (Ed. Raghavendra A.S.) Cambridge University Press, UK: 250-263.

Pollinere J.P., 1996. Introduction to the study of genetical selection of *Hevea brasiliensis*. Tropical Abstracts 21: 6.

Powell D. B. B., 1976. Continuous measurement of shoot extension and stem expansion in the field. *J. Expt. Bot.* 27(101): 1361-1370.

Premakumari D., Panikkar A.O.N. and Shankar S., 1985. Occurence of intraxylar pholem in *Hevea brasiliensis* (Willd. ex Adr. De Juss.) Muell. Arg. *Annals of Botany* 55: 275-277.

Premakumari D. and Panikkar A.O.N., 1988. Studies on the intraxylary phloem and its association with certain growth characters in *Hevea brasiliensis* (Willd. ex Adr. De Juss.) Muell. Arg. *Indian J. Nat. Rubber Res.* 1(2): 41-44.

Premakumari D., 1992. Variability, correlations and path co-efficient analysis for yield in relation to anatomical characters in *Hevea barasiliensis* (Willd. ex Adr. De Juss.) Muell. Arg. Ph.D Thesis, University of Kerala, Trivandrum, India. p.260.

Rajagopal R., Devakumar A.S., Vijayakumar K.R., Varghese Y. A., Rao G.G. and Sethuraj M. R., 1988. Variation in leaf tissue membrane thermostability among clones of *Hevea brasiliensis*. *Indian J. Nat. Rubber Res.* 1:79-82.

Rajagopal V., Kasturi Bai K.V. and Voleti S.R., 1990. Screening of coconut genotypes for drought tolerance. *Oleagineux* 45(5): 215-223.

Rajagopal V., Ramadasan A., Kasturi Bai K.V. and Balasimha D., 1989. Influence of irrigation on leaf water relations and dry matter production in coconut palms. *Irrig. Sci.* 10: 73-81.

Reghu C. P., Mercy M. A. and Lakshmanan R., 2012. Further evaluation and selection of 1981 IRRDB wild *Hevea* germplasm collection in India. *Natural Rubber Research* 25(1): 31-38.

Sam O., Jerez E. and Varela M., 1996. Anatomical characteristics of leaves of potatoes *(Solanum tuberosum* L.*)* and tomatoes (*Lycopersicon escurlentum* Mill.) with different degrees of tolerance to water and heat stress. *Cultivos Tropicales* 17(2): 32-38.

Sethuraj M.R., Potty S.N., Vijayakumar K.P., Krishnakumar A.K., Sanjeeva Rao P., Thapaliyal A.P., Mohan Krishna T., Gururaja Rao G., Chauydhury D., George M.J., Soman T.A. and Rajeswari Meenattoor J., 1989. Growth performance of *Hevea* in the non-traditional regions of India. *Proc. RRIM Rubber Grower's Conference*, 1989.

Shimshi D., Mayoral M.L. and Atsmon D., 1982. Response of water stress in wheat and related species. *Crop Sci* 22:123-128.

Silva Fernandes A. M., Baker E. A. and Martin J. T., 1964. Studies on plant cuticle. 1V. The isolation and fractionation of cuticular waxes. *Ann. Appl .Biol.* 53: 43-58.

Singh R.K. and Chaudhary B.D., 1985. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi. p.318.

Sullivan C.Y., 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurements. in 'Sorghum in the Seventies (Eds.: Rao N.G.P and House L. R.)'. Oxford and IBH Publishing, New Delhi: 247-264. Swaminathan M.S., 1977. Recent trends in plant breeding with special reference to the improvement of the yield potential of rubber. *Journal of the Rubber Research Institute of Sri Lanka: Conference Proceedings* 54: 11-16.

Ticha I., 1985. Ontogeny of leaf morphology and anatomy. In: 'Photosynthesis During Leaf Development (Ed. Zdenek Sestak)'. Dr. W. Junk Publishers, Dordecht: 16.

Trinov I.A., 1996. Effect of drought on the roots of fruit crops. *Sadovodsto i Vinogradarstvo* 5-6: 7-10.

Vijayakumar K.R., Dey S.K., Chandrasekhar T.R., Devakumar A.S., Mohankrishna T., Rao S.P. and Sethuraj M.R., 1998. Irrigation requirement of rubber trees (*Hevea brasiliensis*) in the subhumid tropics. *Agrl. Water Manag.* 35: 245-259.

Yang- Sang Jin, Lee - Soonwon, Ryu - Ountta, Yoon-Tae Myung, Kim - Jonbo, Yang - S.J, Lee - S.W., Ryu O.H, Yoon T.M. and Kim J.H., 1996. Effect of water stress during shoot growing period on growth of apple trees. *RDA - Journal of Agr. Sci, Soil and Fert.* 38(1):435-439.

Varghese Y.A., Abraham S.T., Mercy M.A., Madhavan J., Reghu C.P., Rao G.P., Ammal S. L., Idicula S. P. and Joseph A., 2002. Management of the 1981 IRRDB germplasm collection in India. IRRDB Joint Workshop on Plant Breeding, Agronomy and Socio-economics, August 28-September 7, 2002, Malaysia / Indonesia. p. 20.

Zamski K. and Tsivion Y., 1977. Translocation in plants possessing supernumerary phloem. 1. 14C assimilates and auxin in the internal phloem of tobacco (*Nicotiana tabacum* L). *Journal of Experimental Botany* 28: 117-126.