Pot Culture Experiment on Screening for Tolerance to Drought in a Set of Wild *Hevea* Germplasm

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India received 4,548 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 indicated 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. For identifying the drought tolerance potential of this germplasm collection, a pot culture experiment was conducted at RRII using a set of 16 wild accessions along with the check clones RRII 105, RRII 208, RRIM 600 and Tjir 1. Data recording on growth and drought related morphological, physiological and anatomical parameters was done for assessing the clonal response of these accessions to drought stress. Plant height, girth, number of whorls, number of leaves, distance between whorls and petiole length were the growth parameters studied. As a physiological parameter, after noon leaf water potential (LWP) was recorded during the summer months after withholding irrigation and drought related anatomical parameters studied were xylem characteristics and intra-xylary phloem in twigs. Morphological characterization based on drought sensitive parameters such as leaf size, appearance of leaf surface, presence or absence of wax coating on leaf surface, leaf yellowing and senescence and vigor of plant was done and mortality rate among the accessions was assessed for identifying the potential drought tolerant accessions. 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.

Key Words: *Hevea* germplasm, Drought tolerance, Characterization

India received 4,548 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. Genetic diversity in this collection is closely related to the geographical provenance of the accessions (RRII, 2002) and considerable genetic variation among the accessions from Acre, Rondonia and Mato Grosso provenances has been reported by Varghese *et al.,* 2002. 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.

Materials and Methods

A pot culture experiment was conducted at Rubber Research Institute of India, Kottayam using 16 wild *Hevea* germplasm accessions of 1981 IRRDB expedition along with check clones RRII 105, RRII 208, RRIM 600 and Tjir 1 (Table 1) for identifying the potential drought tolerant accessions. The plants were grown in cement pots of size 2' height and 1' diameter in a Completely Randomized Design with five plants per accession, during 2003-2004. Data recording on morphological and physiological parameters related to drought tolerance was conducted during the pre- and post-summer periods and anatomical observations on primary xylem points and intra-xylary phloem were studied using twig samples. Twig samples of one year growth were collected from two trees per clone. 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 intra-xylary phloem points (IXP) were quantified, and diameter of twigs were recorded and compared. Five sections per twig were observed for recording the characters.

Plant height, girth, number of whorls, number of leaves, internodal length and petiole length were the morphological parameters studied and afternoon leaf

Table 1. The list of wild accessions selected for the study along with the check clones

Accession	Accession	Accession	Check clones
MT 81	MT 4242	RO 1520	RRII 105
MT 200	MT 3714	RO 217	RRII 208
MT 1623	MT 60	AC 462	RRIM 600
MT 1681	MT 48	AC 441	Tjir 1
MT 1631	MT 1584		
MT 1710	MT 52		

water potential after withholding irrigation was used as a drought related physiological parameter. Morphological characterization based on drought sensitive parameters such as leaf size, appearance of leaf surface, presence or absence of wax coating on leaf surface, leaf yellowing and senescence and vigor of plant was done and mortality rate among the accessions was assessed for identifying the potential drought tolerant accessions. The clonal response of these accessions to drought stress was assessed to identify the potential drought tolerant wild accessions.

Results and Discussion

The list of wild accessions selected for the study is shown in Table 1. While assessing the variability among the accessions for morphological parameters during the pre stress period, wide variability was observed among the accessions for all the characters studied. MT 1623 showed superiority in growth and vigour during the pre stress period, and among the check clones, RRIM 600 and RRII 208 were the superior clones (Table 2). In the post stress period also (Table 3), MT 1623 showed the maximum girth whereas the total foliage production was more in MT 200. Among the check clones RRIM 600 showed the superiority over the other clones. Analysis of data from the various field trials, and preliminary observations on sets of accessions from the nurseries, has revealed similar variability and significant differences among the accessions for most of the agro-morphologic traits, bark structural characters and juvenile yield indicating the scope for selection of accessions with desirable characters (Annamma *et al.,* 1989; Abraham *et al.,* 1992, 2000; Madhavan *et al.,* 1993; Mercy *et al.,* 1993, 1995, Rao *et al.,* 1996, 2008). 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 (Suma *et al.,* 2006). In *Hevea,* growth and yield are affected under drought conditions and hence clones with good growth and vigor in the early growth stage are highly preferred for drought-affected areas. There are several earlier reports indicating the importance and usefulness of morphological characters like plant height, basal diameter, number of leaves, leaf area etc. in water stressed or drought condition (De Conceicao *et al*., 1986). These characters give an indication of the general vigour of the genotype and hence studying these characters at the juvenile stage itself holds some importance.

Superiority in terms of girth increment and leaf water potential during the post stress period was high for MT

Table 2. Range and mean of growth characters in the pre stress period

						Check clones					
Characters	Min.	Accession	Max.	Accession	G. Mean	RRII 105	RRIM 600	Tjir 1	RRII 208	CD	VR
Height (cm)	83.8	MT1584	164.6	MT60	112.6	94.4	173.2	144.6	139.8	44.27	2.83**
Girth (cm)	4.5	MT1631	6.0	MT1623	5.24	4.5	5.8	5.6	5.30	–	NS
No. of whorls	1.0	MT1631	2.6	MT1623	1.89	1.5	2.6	1.8	2.75	0.88	3.16**
No. of leaves	8.0	MT 1631	27.0	MT200	19.27	18.0	34.6	27.4	27.5	10.8	3.59**
Inter nodal length (cm)	7.25	MT1584	28.38	MT1623	18.57	15.0	13.45	18.0	11.11	5.29	8.67**
Petiole length (cm)	8.88	MT60	23.38	MT3714	14.67	8.88	12.42	11.78	14.29	4.21	5.38**

** Significant at 1%

Table 3. Range and mean of growth characters in the post stress period

						Check clones					
Characters	Min.	Accession	Max.	Accession	G. Mean	RRII 105	RRIM 600	Tjir 1	RRII 208	CD	VR
Height (cm)	102.0	MT 1584	181.0	MT60	140.0	120.2	211.5	156.0	149.0	41.58	3.19**
Girth (cm)	4.38	MT1631	6.3	MT1623	5.35	4.6	6.3	5.6	5.3	0.94	3.33**
No. of leaves	11.4	MT 1710	38.25	MT200	21.86	23.0	40.75	29.6	26.25	12.14	3.0**

** Significant at 1%

accessions (Table 4). In an early evaluation of wild *Hevea* germplasm for drought tolerance based on growth and dry matter production, Mercy *et al.* (2006) has reported similar growth difference among the accessions. Check clones RRII 208 and Tjir1 did not show any girth increment during this period, whereas it was highest for RRIM 600. With respect to afternoon LWP, some of the MT accessions showed higher LWP than the check clones and among the checks it was high for RRIM 600. An assessment on mortality rate among the 16 accessions indicated 100 per cent survival of two accessions *viz.*; MT 1623 and MT 1681(Table 5). Among the check clones, RRII 208 and RRIM 600 were having less mortality, whereas in the clone RRII 105 it was 100%. Following this, girth increment of accessions with less than 50% mortality was assessed over a summer period (Table 6) 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. Among the check clones

Table 4. Superior accessions in terms of girth increment and AN Leaf water potential in the post stress period

Girth increment (%)	AN leaf water potential (-Mpa)
MT 3714 (8.16)	MT 3714 (-2.27)
MT 81 (6.00)	MT 1631 (-2.4)
MT 1623 (5.00)	MT 48 (-2..67)
MT 4242 (4.17)	MT 1681(-2.93)
MT 1584(4.08)	MT 1584 (-3.06)
RRII 208 (0)	RRII 208 (-3.07)
Tjir 1 (0)	Tjir 1 (-3.34)
RRIM 600 (8.62)	RRIM 600 (-2.8)
RRII 105 (2.22)	RRII 105 (dried)
VR= NS	VR=18.73**CD= 0.35

** Significant at 1%

Table 5. Assessment on mortality rate in the accessions

Accession	Mortality rate (%)	Accession	Mortality rate (%)
MT 1623	0	AC 462	60
MT 1681	0	MT 3714	60
MT 81	20	AC 441	80
MT 200	20	RO 1520	80
RO 217	20	MT 60	80
MT 52	40	MT 1631	80
MT 4242	40	RRII 208	20
MT 48	40	RRIM 600	40
MT 1584	40	Tjir 1	60
MT 1710	60	RRII 105	100

Table 6. Girth increment in accessions with less than 50% mortality over a summer period

Accession	Girth increment (%)
MT 81	60.38
MT 200	57.89
MT 1681	50
MT 4242	50
RO 217	44.74
MT 1623	41.27
MT 1584	21.57
MT 52	14.58
RRII 208	34.53
Tjir 1	38.10
RRIM 600	79.84
RRII 105	0
VR= 3.03**	CD=24.45

** Significant at 1%

RRIM 600 recorded the highest girth increment and the clone RRII 105 was totally affected. Genetic level difference in response towards drought tolerance has been previously reported by Nair *et al*. (2005), while conducting laboratory screening in a set of 200 wild accessions for rapid screening of germplasm accessions for drought tolerance using the chlorophyll fluorescence technique. In an attempt to rapidly screen 3073 *Hevea* germplasm lines for intrinsic drought tolerance traits, using drought related morphological parameters during summer months, there was difference in response to drought tolerance (unpublished data). Previous studies using wild germplasm accessions have revealed genetic difference among the accessions for drought related biochemical parameters (Mercy *et al.,* 2005) and cell membrane stability (Mercy *et al.,* 2000). Similarly, genotypic difference among the wild accessions for drought related leaf and bark anatomical characters are also reported earlier (Mercy *et al.,* 2004).

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). The wild accessions showed wide variation with respect to PXP, IXP and diameter of twigs (Table 7). 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,

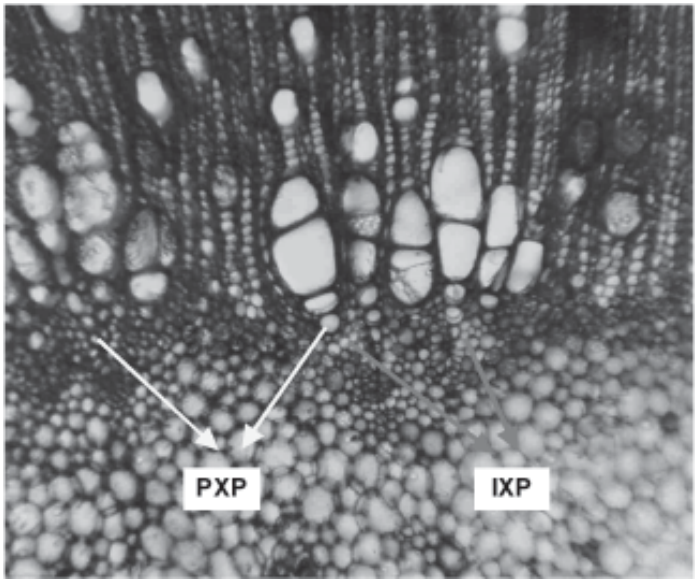

Fig. 1: Transverse section of a twig showing primary xylem and intraxylary phloem in *Hevea brasiliensis* (PXP–primary xylem points; IXP–intraxylary phloem)

Table 7. Potential accessions for structural traits along with twig diameter

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

** Significant at 1%

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.

Occurrence of an internal phloem (intraxylary) in the xylem tissue is reported in some species of Euphorbiaceae (Metcalf and Chalk, 1950). In *Hevea*, the occurrence of adaxial (medullary) phloem associated with protoxylem was noticed first, during the studies on periodicity of cambial activity 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. Moreover, quantitative analysis of conductive tissues is observed to show a close correspondence of wood structure in relation to habitat (Carlquist and Debuhr, 2008). In the present study, the high variability for IXP

Table 8. Morphological characterization based on drought sensitive parameters

Accn.	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

observed is in concurrence with earlier observations (Mary, 2004). The wild accession MT 1623, which showed superiority for most of the characters in this study, also exhibited significantly superior values for IXP, compared to the proven drought tolerant clones RRIM 600 and RRII 208 suggesting the use of this trait for selection towards potential drought tolerant accessions. The linear relationship observed for IXP tissues and growth as well as the possible activation of this tissue under conditions of stress assumes significance in this context.

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 8) for identifying the potential drought 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. Field level screening of the wild accessions conducted in the drought prone area in Dapchari, Maharashtra, also has proved genotypic difference among the accessions towards drought tolerance (Mercy *et al.,* 2009).

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). As the genetically divergent germplasm accessions are an excellent repository of various useful traits including stress tolerance, systematic screening of wild germplasm for drought and cold tolerance with the ultimate objective of developing location specific clones for non-traditional rubber growing areas holds much importance.

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