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Evaluation of wild *Hevea* germplasm for drought tolerance based on leaf and bark anatomical characteristics

M. A. Mercy, L. C. Babu* and Y. Annamma Varghese

Rubber Research Institute of India, Rubber Board P. O., Kottayam- 686 009, Kerala, India.

Abstract

Commercial plantations of natural rubber, *Hevea brasiliensis* are normally raised under rainfed conditions. In India, extension of rubber cultivation to non- traditional and marginal areas have necessitated selection/ development of clones capable of withstanding drought situation along with other environmental stresses prevailing in these non-traditional rubber growing areas. Since the extent of genetic variability for such traits is very low in the current breeding pool, now the focus is on the wild *Hevea* germplasm collection, to generate variability for drought tolerant traits. The present investigation has been made for evaluating certain wild accessions employing a set of drought related leaf anatomical traits viz., stomatal density, leaf thickness, diameter of midrib, thickness of palisade and mesophyll tissue, number of palisade cells per unit length and bark characters viz., total bark and soft bast thickness, total number of latex vessel rows, proportion of soft bast, and proportion of latex vessel rows in it, in an early growth phase. Wide variability exists for all the traits studied, but was not statistically significant for the bark anatomical traits. Among the leaf anatomical parameters studied, stomatal density, thickness of mesophyll tissue, palisade cell number/ unit length etc. are the potential traits useful for short-listing accessions for drought tolerance in the early growth phase.

Key words: Rubber, drought tolerance, germplasm accessions, anatomical parameters

Introduction

Hevea brasiliensis (Willd. ex. Adr. de Juss.) Muell. Arg. is the commercial source of natural rubber and the species is well suited to equatorial region with plenty of well distributed rainfall and minimum fluctuations in temperature and throughout the world, rubber plantations are raised under rain fed conditions. To cope up with the increasing global demand for natural rubber and considering the limited scope of expansion of the crop in its favoured traditional belt, attempts have been made to extend this tree to marginal areas with varied climatic constraints (Sethuraj *et al.,* 1989) such as prolonged soil moisture stress and high ambient day temperatures in the western and eastern India. Soil and atmospheric drought and high temperature are major environmental factors limiting growth and yield in *Hevea* even in the traditional areas. Early detection of stress resistant traits in the available genetic resources is very much useful in any crop, especially in a perennial crop like rubber.

In *Hevea,* the narrow genetic base of the present day cultivated clones resultant of the small genetic stock introduced by Sir Henry Wickham and the unilateral selection for yield over the years limited the availability of sufficient genetic variability for various biotic and abiotic stresses including drought. Several investigations on drought tolerance in crop plants have led to the observation that wild relatives of cultivated species are drought tolerant (Shimshi *et al.,* 1982). Rosenow *et al.* (1983) reported better performance of wild cotton germplasm than commercial germplasm under water stress condition. In India, a large collection of over 4500 wild accessions resultant of an expedition organized by the International Rubber Research and Development Board (IRRDB) in 1981 in the Amazon forests, the primary center of origin of the crop, are being conserved, characterized and evaluated. Since, the exploration covered a wide range of agro climatic areas in the three Brazilian states Acre (AC). Rondonia (RO) and Mato Grosso (MT), this material is a reservoir of many valuable

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Email : mamercy@rubberboard.org.in

** College of Forestry, Kerala Agricultural University, Thrissur, Kerala.*

genes especially those conferring resistance to various biotic and abiotic stresses. *Hevea*, being a perennial tree species, subjecting all the accessions to actual drought conditions and selecting the ones least affected by drought is time consuming.

Crop plants adapt to stress conditions by the intervention of several inductive morphological, anatomical, physiological and biochemical mechanisms (Hanson, 1980; Kramer, 1983). Various reports on 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 include those by Streitberg (1975), Rajagopal *et al.* (1990), Sam *et al.* (1996), Harvey *et al.* (1997), Mei Xiuying *et al.* (1998) and Kumar *et al.* (2000). In a comparative bark anatomy study of drought tolerant and drought susceptible *Hevea* clones, Premakumari *et al.* (1993) observed significant difference in characters such as height and width of phloic rays, height: width ratio of phloic rays, proportion of soft bast to total bark thickness and proportion of latex vessel rows in the soft bast to total number of latex vessel rows. With this background, the present study was undertaken to evaluate the usefulness of some of the leaf and bark anatomical traits for screening of germplasm for drought tolerance and to assess the genetic variability among the selected wild *Hevea* accessions for drought related leaf and bark anatomical characters at an early growth phase.

Materials and Methods

Wild germplasm material conserved in the source bush nursery of Central Experimental Station (CES) of Rubber Research Institute of India (RRII) constituted the base material for the present study. Ten accessions were selected based on preliminary studies conducted among 450 accessions for growth parameters during summer months. They were multiplied along with the standard clones viz., RRII 105 (popular high yielding clone), RRIM 600 (known drought tolerant clone) and Tjir 1 (known drought susceptible clone) and established in polythene bags of size lay flat dimension of 55 cm length, 25 cm width and 400 gauge thickness. At the age of 10 months, physiologically mature leaves from the top whorl were collected for leaf anatomical parameters. The leaf samples collected were preserved in 1:1:18 formalin-acetic-alcohol (FAA). Leaf bits of one cm^2 size covering the midrib and either side of the lamina, were prepared and hand sectioned at cross sectional plane and stained using Safranine stain and observed in Aristoplan trinocular research microscope equipped with camera. Thickness of palisade tissue (mm), thickness of mesophyll tissue (mm), mean number of cells in unit length of palisade layer, leaf lamina thickness (mm) and leaf vein (midrib) diameter (mm) were recorded from the cross sections of the leaves. Stomatal density was determined as the number of stomata per mm^2 in the abaxial surface of leaf lamina from epidermal peelings taken from abaxial surface, separated by boiling the leaf bits in 60% nitric acid with a pinch of potassium chlorate and the stomatal count was expressed as number of stomata per square mm.

Bark samples for anatomical studies were collected from plants at a height of 15 cm from the bud union. Sections, 60 μm thick, were cut using a sledge microtome. Radial longitudinal bark sections, stained using Sudan III stain was observed using a compound microscope to record the bark anatomical characters viz., total number of latex vessel rows (TLVR), number of latex vessel rows in the soft bast, total thickness of bark (mm) and thickness of soft bast (mm). Data were subjected to analysis of variance and based on the significance of F value, accessions were ranked following Duncan's multiple range test. Individual performance of the accessions was assessed by summing up of the rank values obtained for each character, based on the parametric relationship of these characters to drought tolerance (Singh and Choudhary, 1985).

Results and Discussion

a. Leaf anatomical characters

The green leaves of plants are photosynthetically active organs, which are able to store absorbed solar energy in reduced organic compounds. Leaf structural characters influence the net leaf photosynthesis to a large degree and thus cause great differences in light use efficiency. Stomatal pores, which are minute intercellular openings bounded by two kidney-shaped guard cells plays significant role in the exchange of water vapour, CO_2 and O_2 between the internal and external atmosphere of the leaves ultimately controlling the photosynthetic efficiency of the plants (Bolhar- Nordenkampf and Draxler, 1993). It is the response of the stomata, through the regulation of stomatal conductance, to various environmental parameters that determine the environmental demand on the leaf to supply water for evaporation (Rachel and Baker, 1983). Stomatal density is thus an important parameter, determining the water use efficiency of the leaf and in turn, that of the plant. Stomatal characteristics are species specific and may vary according to the habitat, leaf age and insertion level (Ticha *et al.*, 1982). Stomata in *H. brasiliensis* belong to the paracytic type (Panikkar, 1974) and in the wild accessions also the same type of stomata was observed. In all the wild accessions studied, as well as in control, stomata were present only in the lower epidermis of the leaves. This was in agreement with the earlier reports of Rao (1963) in elite cultivars of *H. brasiliensis*. The stomatal density ranged from 277 to 481 (Table 1) with an average density of 376. Low stomatal density recorded in the accessions AC 446 (277) and MT 938 (296) indicate their drought tolerance capacity. In general, the stomatal density was lower in the wild accessions compared to that of control clones RRIM 600 (357), Tjir 1(447) and RRII 105 (427). Since higher number of stomata per leaf surface would increase the transpirational water loss for crops growing under rainfed condition, a low number of stomata per plant is found to be desirable (Jones, 1977) and hence a comparatively less number of stomata present among the wild *Hevea* germplasm is advantageous. The importance of stomatal studies in breeding for drought tolerance in crop plants has been reviewed by Jones (1979). Since stomatal frequency is influenced by light, temperature and water stress, Ciha and Brun (1975) suggested that stomatal sensitivity to moisture stress should be taken as an indicator of drought response of plants, rather than stomatal frequency. Fairly high number of stomata per unit leaf area and the poor stomatal sensitivity may be the reasons for the drought susceptibility of Tjir 1. Genetic variability has been demonstrated for various stomatal characteristics by Clarke and Smith (1986) as observed for stomatal density in the present study.

Internal architecture of the leaf is complex and varies considerably from species to species. Leaf structure also has a critical role in the water relations of the plant. In rubber, where vegetative growth is important it is necessary to know the relationship between leaf area, leaf structure and leaf photosynthesis (Swaminathan, 1977). Important leaf structural traits contributing directly or indirectly to the water relations and gaseous exchange of the plant were recorded in this study viz., thickness of the lamina and midrib, thickness of the palisade and mesophyll layers and number of cells in unit length of palisade layer. All these characters showed significant genetic differences among the wild accessions studied. Similar results of significant clonal differences were reported by Gomez and Hamzah (1980) for all these characters in *Hevea* which is in concurrence with this study.

Thickness of lamina is a character of great importance due to its association with photosynthetic capacity. Leaf thickness increases, along with the leaf area, which involves both cell division and cell enlargement (Ticha, 1985). There is plenty of experimental evidence to suggest that both adaptive and genetic differences in the rate of photosynthesis per unit leaf area are associated with differences in leaf thickness (Charles, 1983). Changes in leaf thickness might be expected to affect movement of CO_2 in the gaseous phase. There appears to be concomitant changes in the anatomy (stomatal density and distribution) and structure (mesophyll cell size) of the leaf with changes in leaf thickness, which tend to minimize the effects of these changes on the photosynthetic functioning of the leaf (Charles, 1983). Leaf thickness is a character, which changes itself to adapt to the available light intensity for

Table 1 . Variability for leaf structural characters of selected accessions of *Hevea brasiliensis*

Accessions	No. of stomata per mm^2	Leaf thickness (mm)	Midrib diameter (mm)	Palisade tissue thickness (mm)	Mesophyll tissue thickness (mm)	Palisade no. per unit length
AC 1044	451^{ab}	123.93^{abc}	438.90^{a}	54.63^{bcde}	104.27^{abcd}	34.26^{ab}
MT 55	390^{abcd}	105.77^{fg}	310.67^{g}	48.04^{efg}	86.35^{ef}	28.60^{de}
AC 446	277^{d}	117.90^{bcde}	371.00^{bcd}	47.73^{efg}	84.95^{f}	30.22^{bcd}
RRIM 600	357^{abcd}	123.77^{abc}	352.20^{defg}	51.36^{cdef}	105.79^{abc}	35.24^{a}
Tjir 1	447^{ab}	129.83^{a}	406.80^{ab}	64.65^{a}	114.52^{a}	24.29^{f}
MT 41	333^{bcd}	104.23^{g}	360.73^{cde}	44.14^{fg}	82.67^{f}	33.51^{abc}
MT 76	357^{abcd}	127.83^{a}	346.48^{defg}	59.37^{abc}	102.78^{abcd}	31.19^{abcd}
MT 66	481^{a}	109.43^{efg}	419.53^{a}	48.31^{efg}	88.50^{def}	34.59^{a}
MT 938	296^{cd}	121.50^{abcd}	311.90^{fg}	42.42^{g}	101.38^{abcde}	33.47^{abc}
AC 650	331^{bcd}	114.43^{cdef}	355.18^{def}	50.43^{defg}	95.65^{bcdef}	29.76^{cde}
AC 652	349^{abcd}	127.70^{a}	312.93^{fg}	62.31^{ab}	109.79^{ab}	33.40^{abc}
RRII 105	427^{abc}	127.27^{ab}	400.20^{abc}	58.08^{abcd}	107.26^{abc}	32.38^{abcd}
AC 728	394^{abcd}	113.73^{def}	318.67^{efg}	51.64^{cdef}	92.47^{cdef}	26.04^{ef}
Mean	376	119.03	361.94	52.5	98.18	31.30

Any two means having a common letter are not significantly different best light interception. Plants growing in weak light conditions have large and thin leaves (Ishii, 1998) and increased leaf thickness and thick cuticle are some of the xeromorphic characters. Leaf lamina thickness of the wild accessions studied varied from 104.23 to 129.83 mm and the mean value of the character was 119.03 mm. Among the wild accessions the leaf lamina was the thickest in MT 76 (127.83 mm) followed by AC 652 (127.70 mm). This shows the genetic potential of these accessions for drought tolerance, as the increased leaf thickness is associated with increased parenchyma cell size and lowered stomatal frequency as reported by Kumar *et al.* (2000) in drought tolerant coconut cultivars. Increase in leaflet thickness causes decrease in the ratio of the external surface to its volume (Oppenheimer, 1960).

The midrib was very prominent and abuts the lower surface. It was semicircular in the cross sectional view. This was in agreement with the earlier report of Panikkar (1974) in Wickham clones of *Hevea.* The thickness of the leaf midrib assumes importance in relation to the translocation of photosynthates from the sites of their production and its role in water conduction. Diameter of midrib ranged from 310.67 - 438.9 mm with a mean of 361.94 mm. It was the minimum in MT 55 (310.67 mm) followed by MT 938 (311.90 mm) and AC 652 (312.93 mm). Among the control clones, the drought tolerant clone RRIM 600 showed a significant reduction in midrib diameter (352.2 mm) compared to the drought susceptible clone Tjir 1 (406.8 mm) and the clone RRII 105 (400.2 mm) which is drought tolerant with respect to summer yield but susceptible with respect to growth. The lower midrib diameter in majority of the wild accessions as that in RRIM 600, highlights the suitability of these accessions in drought prone areas, where the water loss from the plant through the midrib is minimum, but at the same time the translocation of photosynthates is not much affected.

Mesophyll is a specialized photosynthetic tissue consisting of the palisade and spongy tissues. Both these tissues offer distinct functions in the plant's activities. The palisade cells function primarily for assimilation due to the presence of chloroplast in them, whereas the spongy parenchyma besides performing the function of assimilation performs the function of aeration of the interior of the leaf. The palisade parenchyma of the leaf of *Hevea* is composed of a single row of cells, which are elongated and lie so closely together that no intercellular spaces are formed. Even though spongy parenchyma has a much larger intercellular space than the palisade tissue, the palisade tissue has a larger free surface exposed to the internal atmosphere. The ratio of internal to external surface is strongly and positively correlated with the rate of transpiration (Turrell, 1944). Moreover, palisade cells in sun leaves are closely associated with the adaxial epidermis and hence water transport towards the epidermis is much higher through the palisade tissue than the spongy parenchyma. There will be a reduction in photosynthetic rate as a result of a decrease in surface area of palisade parenchyma/ unit area (Kumar *et al.*, 2000). Hence, the low palisade tissue thickness found in the wild accessions MT 938, MT 41, AC 446 and MT 55 and in the control clone RRIM 600 is advantageous for imparting drought tolerance. Spongy parenchyma consists of rounded or irregular cells, which result in the formation of large intercellular spaces between them. The upper and lower epidermis, contains no chlorophyll in the case of *Hevea* (Bobilioff, 1923). This characteristic structure of the leaves seen in the wild *Hevea* is suggestive of the fact that these wild clones would have developed in their original habitat in abundant sunlight. These anatomical characters are by and large fixed early in leaf development and well before full expansion of the lamina so that any later transfers between light environments have limited effect on leaf structure (Terasimha and Hikosaka, 1995). The mesophyll tissue thickness of the wild accessions was maximum in AC 652 (109.79 mm) followed by AC 1044 (104.27mm) and MT 76 (102.78mm). Increased parenchyma cell size leads to reduction in the intercellular space/unit area. This will help in reducing the water conductance towards epidermis thereby reducing the transpirational rates and maintaining high water potentials. Hence, wild accessions with more mesophyll tissue thickness are preferred under drought prone areas. When we consider the thickness of spongy parenchyma, on the basis of thickness of mesophyll tissue, the wild accession MT 938 was having the highest thickness indicating the reduced intercellular space/unit area in it, thereby maintaining high water potential by reducing the rate of transpiration.

Number of palisade cells per unit length varied from 24 - 35 with a mean of 31 cells. Among the wild accessions, the maximum palisade cell number was in MT 66 (35) followed by AC 1044 (34) and the lowest was recorded in AC 728 (26). The control clones RRIM 600 and RRII 105 recorded higher number of palisade cells (35 and 32 respectively) than the mean value (31), whereas in Tjir 1, it was less (24) than the mean value. The greater number of cells in a cross section of sun leaves results in more chloroplasts per unit area in sun as compared to shade developed leaves, although the chloroplasts in shade leaves are typically larger and contain more chlorophyll (Terasimha and Hikosaka, 1995). This may be one of the reasons for the drought susceptibility of Tjir 1. The wide variability expressed in the wild germplasm ensures efficient and successful selection of superior wild accessions with such desirable traits for crop improvement. According to earlier reports, drought tolerant traits such as greater leaf lamina thickness, low stomatal density, stomatal conductance, rate of transpiration and photosynthesis along with higher content of epicuticular wax and leaf water potentials are some of the useful parameters in identifying a drought tolerant clone in a screening programme (Kasturi Bai, 1993; Kumar *et al.*, 2000; Mercy, 2001).

(b) Bark anatomical characters

Yield in *Hevea* is a clonal characteristic influenced by environmental factors and significant clonal variation in the summer yield drop has been reported by Sethuraj and George (1976). Rubber yield is mainly determined by the total latex vessel rows present in the bark and hence bark anatomical characters have special importance on yield. The importance of structural parameters such as bark thickness, number of latex vessel rows and diameter of latex vessels as yield contributing factors are well known (Gomez *et al.*, 1972). Earlier studies on the relationship between various bark structural characters and yield in *H. brasiliensis* had revealed that the number of latex vessel rows was the most important single factor related to yield (Premakumari *et al.*, 1985). This was reported to vary greatly with the age and type of the clone (Riches and Gooding, 1952). Characters like total bark thickness and number of latex vessel rows of *Hevea* clones at the juvenile stage have been reported as clonal characteristics (Ho *et al.*, 1973).

Bark structural traits studied include total bark thickness, proportion of soft bast region, total latex vessel rows, and proportion of latex vessel rows in the soft bast. This study has revealed wide variability for these characters (Table 2). The total bark thickness varied from 1.277 - 1.623 mm with a mean of 1.398 mm. The highest was in the wild accession MT 41(1.623 mm) followed by MT 66 (1.513 mm) and the lowest was in AC 650 (1.277 mm). There was no significant genotypic difference for this character, and majority of the wild accessions and the selected control clones had comparable values. Soft bast thickness also did not show any significant genotypic difference, and ranged between 0.57 - 0.77 mm with a mean value of 0.66 mm. The soft bast thickness was maximum in the wild accessions AC 446, AC 1044, and MT 66 and the accessions MT 55 and MT 938 recorded lowest values. The clones RRIM 600 (0.69 mm) and RRII 105 (0.67 mm) had comparable soft bast thickness, whereas in Tjir 1, it was higher (0.73 mm).

Premakumari (1992) had reported the proportion of soft bast to differ significantly in drought tolerant and susceptible Wickham clones of rubber. The proportion of soft bast and proportion of TLVR in the soft bast zone in *Hevea* clones gives an indication of their response to drought, as confirmed by Premakumari *et al.* (1993) in studies on drought susceptible and tolerant Wickham clones. Significantly higher proportion of soft bast and TLVR in soft bast was noticed in drought susceptible

Table 2. Variability for bark structural characters of selected accessions of *Hevea brasiliensis* at juvenile stage

Genotypes	Total thickness of Bark (mm)	Thickness of soft bast (mm)	Proportion of soft bast thickness (%)	Total no. of LVR	No. of LVR in soft bast	Proportion of LVR in the soft bast (%)
AC 1044	1.383^{ab}	0.72^{ab}	51.79^{ab}	3.00^{a}	2.00^{a}	66.67^{b}
MT 55	1.340^{b}	0.57^{b}	42.68^{bc}	2.33^{ab}	1.67^{a}	72.22^{ab}
AC 446	1.360^{ab}	0.77^{a}	56.53^{a}	3.00^{a}	2.00^{a}	72.22^{ab}
RRIM 600	1.307^{b}	0.69^{ab}	52.60^{ab}	3.00^{a}	2.00^{a}	66.67^{b}
Tjir 1	1.493^{ab}	0.73^{ab}	49.17^{abc}	3.00^{a}	2.00^{a}	66.67^{b}
MT 41	1.623^{a}	0.67^{ab}	41.64^{bc}	3.33^{a}	2.33^{a}	66.67^{b}
MT 76	1.370^{ab}	0.63^{ab}	46.22^{abc}	2.77^{a}	2.10^{a}	76.76^{ab}
MT 66	1.513^{ab}	0.71^{ab}	47.52^{abc}	2.67^{a}	2.00^{a}	72.68^{ab}
MT 938	1.465^{ab}	0.57^{b}	38.93^{c}	1.33^{b}	1.33^{a}	100.00^{a}
AC 650	1.277^{b}	0.60^{ab}	47.28^{abc}	3.23^{a}	2.23^{a}	68.77^{b}
AC 652	1.370^{ab}	0.60^{ab}	44.92^{abc}	2.43^{ab}	1.67^{a}	70.20^{b}
RRII 105	1.347^{ab}	0.67^{ab}	49.61^{abc}	3.00^{a}	1.89^{a}	6300^{b}
AC 728	1.323^{b}	0.67^{ab}	50.81^{ab}	2.77^{a}	2.10^{a}	78.79^{ab}
Mean	1.398	0.66	47.67	2.76	1.95	72.41

Any two means having a common letter are not significantly different

Hevea clones. However, the differences were not significant in the present study, which may be due to the juvenile growth phase where such differences were not established. Similar to the results in this study, Nazeer *et al.* (1992) could not get statistical significance for the characters, total bark thickness and total number of latex vessels in a set of 15 Wickham clones, in the immature growth phase, even though they observed slight numerical differences between the clones.

However, the variation noticed among wild accessions for these characters in the present study gives an indication of the extent of variability for drought tolerance in the wild germplasm. Varghese *et al.* (1988) have reported wide variability in young wild germplasm for the characters total bark thickness and total number of latex vessel rows. All these reports are in conformity with the results of this study. Proportion of soft bast thickness ranged from 38.93 to 56.53% and the mean proportion was 47.67%. The accessions AC 446 and MT 938 differed significantly for this character. Accessions AC 446 and AC 1044 were having highest proportion of soft bast (56.53 and 51.79% respectively) and the lowest proportion was in MT 41 (41.64%). All the 3 control clones RRIM 600, RRII 105 and Tjir 1 had more or less similar soft bast proportions (52.60, 49.61 and 49.17% respectively). High proportion of soft bark was reported to be related to the drought susceptibility of the clones and vice versa (Premakumari, 1992) and hence MT 41 is more suitable than the remaining accessions in a drought prone area. Total number of latex vessel rows (TLVR), one of the most important yield determining bark structural traits in *Hevea*, was in the range of 1.33 – 3.33 with a mean of 2.76. The highest number of latex vessel rows was in MT 41 (3.33) while the lowest was in the accession MT 938 (1.33). The significant genotypic difference was seen only in MT 938 and the remaining wild accessions had no significant genotypic difference. But the wild accessions AC 1044, AC 446, MT 41 and AC 650 had similar TLVR as in the three control clones, RRII 105, RRIM 600 and Tjir 1.

The proportion of LVR in the soft bast region varied from 63 - 78.79% with a mean of 72.41%. The lowest proportion was seen in both the wild accessions AC 1044 and MT 41 (66.67%). The lowest soft bast proportion and the lowest TLVR proportion in the soft bast noticed in the wild accession MT 41 indicate its genetic potential for drought tolerance capacity. The drought tolerance of this accession was confirmed in further studies in drought prone region in North Konkan. The proportion of TLVR in the soft bast region of RRII 105 recorded the lowest value of 63% whereas in RRIM 600 and Tjir 1, this was on par (66.67%). Significant genotypic difference was noticed in MT 938 for this character, where the proportion of latex vessel rows in the soft bast region was 100%.

(c) Identification of superior accessions based on rank sums

Adaptation of a clone to drought tolerance is a cumulative effect of all the useful traits. Hence, a ranking of each accession based on parametric relationships with drought tolerance is useful for knowing the actual worthiness of each accession which is shown in Table 3. The rank sums varied from 43 to 81. The highest rank sum was obtained by the accession AC 652, followed by AC 1044, MT 938, MT 76 and MT 41.

When the accessions differ from each other for different drought related parameters, a ranking like this gives a better understanding of the drought tolerance

Table 3. Ranking of selected accessions of *Hevea brasiliensis* based on parametric relationship with drought tolerance

Accession	TTB	TSB	PSB	TLVR	LVRS	PLRS	NS	LT	MD	PTT	MTT	PN	Rank Sum
AC 1044	7	2	2	7	5	9	2	8	10	8	9	9	78
MT 55	3	9	8	2	8	5	4	2	1	4	3	2	51
AC 446	4	1	1	7	5	5	10	6	8	3	2	4	56
MT 41	10	4	9	10	1	9	7	1	7	2	1	8	69
MT 76	5	6	6	5	3	3	5	10	5	9	8	5	70
MT 66	9	3	4	4	5	4	1	3	9	5	4	10	61
MT 938	8	9	10	1	10	1	9	7	2	1	7	7	72
AC 650	1	7	5	9	2	8	8	5	6	6	6	3	66
AC 652	5	7	7	3	8	7	6	9	3	10	10	6	81
AC 728	2	4	3	5	3	2	3	4	4	7	5	1	43

TTB – Total thickness of bark
TSB – Thickness of soft bast
PSB – Proportion of soft bast
TLVR – Total number of latex vessel rows
LVRS – Number of LVR in soft bast
PLRS – Proportion of LVR in the soft bast
NS – Number of stomata
LT – Leaf thickness
MD – Mid rib diameter
PTT – Palisade tissue thickness
MTT – Mesophyll tissue thickness
PN – Palisade number per unit length

potential of individual accession. Such a ranking was adopted in cocoa accessions, while screening drought tolerance among these accessions (Balasimha *et al.*, 1988). The top five accessions AC 652, AC 1044, MT 938, MT 76 and MT 41 with higher rank sums indicate their genetic potential for drought tolerance which can be incorporated in further breeding programmes.

The results of the present study reveal existence of wide genetic variability among the wild germplasm accessions for leaf anatomical parameters such as stomatal density, thickness of the leaf lamina, midrib, palisade and mesophyll layers and the number of palisade cells per unit length, related to drought tolerance. These parameters are useful indices for screening a large number of germplasm materials, considering their importance in imparting drought tolerance, whereas bark structural characters are more reliable in the mature stage. Among the bark structural characters, total number of latex vessel rows possesses the maximum importance because of its direct relationship with yield. Hence, this can be used as a reliable parameter in screening of mature *Hevea* clones with drought tolerance combined with high yield.

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