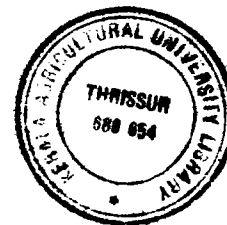


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**GENOTYPIC EVALUATION AND SCREENING FOR
DROUGHT TOLERANCE IN WILD *Hevea* GERMPLASM**

By

M.A. MERCY



THESIS

Submitted in partial fulfilment of the
requirement for the degree of

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Kerala Agricultural University**

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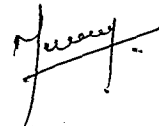
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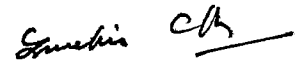


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Certified that this thesis, entitled “**Genotypic evaluation and screening for drought tolerance in wild *Hevea* germplasm**” is a record of research work done independently by **Smt.M.A.Mercy**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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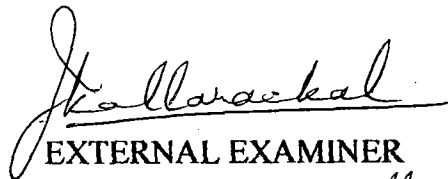
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*to my beloved husband and
sweet little daughter*

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ABBREVIATIONS USED

cm	- centimeter
CRD	- Completely randomized design
DMSI	- Dry matter stress tolerance index
ECW	- Epicuticular wax
Fig.	- Figure
GA	- Genetic advance
GCV	- Genotypic coefficient of variation
g	- gram
H ²	- Heritability (broad sense)
i.e.	- that is
kg	- kilogram
LVR	- Latex vessel rows
m	- meter
mm	- millimeter
MPa	- mega pascal
PCV	- Phenotypic coefficient of variation
s	- seconds
Viz.	- namely
µg	- microgram
µm	- micrometer
Ψ	- water potential

Introduction

INTRODUCTION

Rubber tree (*Hevea brasiliensis* Muell. Arg.) is the only 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. Indonesia, Thailand, Malaysia, China and India are the major countries in the world growing natural rubber. In all these countries, rubber plantations are grown under rainfed conditions. In India, the traditional region of rubber cultivation is the southern part of the west coast extending from Kanyakumari (8° 15' N) to Mangalore (12° 52' N). To cope with the increasing global demand for natural rubber and considering the limited scope of expansion of the crop in its favoured traditional belt, attempts are made to extend its cultivation to agroclimatically marginal areas (Sethuraj *et al.*, 1989). But major environmental constraints are prolonged soil moisture stress and high temperatures prevailing in such marginal areas in the East and West of peninsular India. While soil and atmospheric drought and high temperature are major environmental factors limiting growth and yield in *Hevea* in nontraditional areas, incidence of unexpected droughts often pose problems, even in the traditional area. Severe droughts were experienced in the traditional areas during 1982-83 and 1986-87 (Ouseph, 1987). Understanding of the effects of drought on the crop and evolving drought tolerant clones are critical to realize better productivity in the traditional area and to extend the crop to drought prone nontraditional areas.

Early detection of stress resistant traits in the available genetic resources is useful in any crop, especially in a perennial crop like rubber. But the very narrow genetic base of the cultivated *Hevea* species resulted from the small genetic stock introduced by sir Henry Wickham and the unidirectional selection for yield over years in the cultivated clones limits the availability of wide genetic resources in *Hevea*. Hence for any further crop improvement programme,

widening of this narrow genetic base is essential which can be achieved by introgression of appropriate alien genes from the wild progenitors. Several investigations on drought resistance 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. By proper evaluation and research in these materials, novel candidate genes coding for stress tolerance traits can be identified which can be utilized in genetic improvement programmes (Paroda, 1993).

The wild genotypes 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 good source of genetic variability. Around 5000 genotypes of this wild Brazilian germplasm are being conserved in India. The exploration covered a wide range of agroclimatic areas in the three Brazilian states of Acre (AC), Rondonia (RO) and Mato Grosso (MT). Ong *et al.* (1983) reported the presence of vigorous and high yielding rubber trees in the states of Acre and Rondonia with better quality rubber in Acre. The agroclimatic conditions in Mato Grosso and Acre shows the possibility of selection of genotypes having drought tolerance whereas Rondonia state is predominantly of marshy lands. Hence including genotypes from Mato Grosso and Acre for drought tolerance screening is a right approach in *Hevea* breeding for drought-resistance.

The best method for drought tolerance breeding is to subject the genotypes to drought conditions and select the ones least affected in growth/yield performance. *Hevea*, being a perennial tree species, requires more than 30 years for the above procedure and hence it is not a feasible proposition. Many morphological, physiological, anatomical and biochemical indices have shown their relationship to drought tolerance in earlier studies in many crops including

rubber. Utilising these indices, in *Hevea* seedlings under induced water stress during summer periods, we can effectively screen the germplasm materials within a short period of time to identify genotypes with drought tolerance. The genotypes if any, so isolated can be utilized in further breeding programmes.

With this background, the present study was undertaken with the following objectives,

1. Preliminary screening for drought tolerance in 99 wild *Hevea* germplasm based on cell membrane thermostability and assessing the genetic variability among these genotypes for this trait.
2. Studying the genotypic response and assessing the nature and extent of genetic variability for various physiological characters among the selected genotypes under various levels of induced water stress.
3. Assessing the nature and extent of genetic variability among the selected genotypes for various drought related morphological, biochemical and anatomical characters.
4. Assessing the degree of association among characters studied.
5. Assessing the genetic divergence and clustering the genotypes.
6. Identifying superior genotypes based on rank sum obtained for characters selected on the basis of parametric relationship with drought tolerance.

Review of Literature

REVIEW OF LITERATURE

Crop plants rarely attain their full genetic potential for yield because of the limitations imposed by the environment. Among the various environmental stresses, drought stress is the one, which has deleterious effects on yield of economically important crops. One approach to improve crop performance in water-limited environment is to select for genotypes that have improved growth and yield in this environment. This approach has been proved partially successful, but difficult due to the variability of rainfall and the polygenic nature of drought avoidance and intrinsic tolerance traits. A complementary approach to improve plant performance in water-limited environments involves the identification and selection of traits that contribute to drought avoidance, drought tolerance or water use efficiency. However, most of these traits are complex and our understanding of their interactions and control is limited. Moreover, drought often interacts with other stresses, particularly temperature extremes, high light intensities and with biotic stress, making breeding for drought resistance/tolerance much more complex. Hence, plant responses to water deficit have to be analysed systematically by identifying traits that relate to drought tolerance followed by analysis of the physiological, cellular, biochemical and molecular basis of the trait.

2.1 Electrolyte leakage from cell membranes

The cell membrane is a central physiological site mediating the effect of various environmental stresses on the plant cell. The cell membrane permeability which is usually measured by the electro conductivity measurement of the electrolyte leakage has been widely used as a measure of drought and heat tolerance in sorghum (Sullivan and Ross, 1979), soyabean (Martineau *et al.*, 1979), wheat (Blum and Ebercon, 1981), turf grass (Wallner *et al.*, 1982), potato (Bansal and Nagarajan, 1983; Nagarajan and Bansal, 1986), and in *Hevea*

(Rajagopal *et al.*, 1988). The interrelationship between cell membrane stability and lipid peroxidation for identifying drought tolerant coconut cultivars has been studied by Chempakam *et al.* (1993) who identified that lipid peroxidation affects normal cell functions causing damage to the cell constituents leading to increased cell permeability and leakage. Kurup *et al.* (1993) observed low leaf water potential and high electrolyte leakage in the drought susceptible coconut genotypes. Nair *et al.* (1995 and 1999) studied the effect of heat and drought stress in various *Hevea* clones by a modified electrolyte leakage method and suggested that the method is useful for evaluation of large number of *Hevea* genotypes for tolerance to combination of heat and water stresses. Using a modified protocol Hussain *et al.* (1995) screened heat tolerant and sensitive varieties in *Brassica* for determining membrane thermostability.

Cell membrane stability of leaf tissues and its relationship with drought tolerance in *Arachis* was studied by Deb *et al.* (1996). Evaluating various screening techniques for drought tolerance in wheat (Ashraf *et al.*, 1996) observed cell membrane stability test to be the most reliable and potentially useful one for screening at early growth phase. Measurement of electroconductivity of leaf diffusate was found adequate for rating drought tolerance and to evaluate invisible injury caused by drought or heat stresses in legumes (Grzesiak *et al.*, 1996). In chinese cabbage, Shao Bo *et al.* (1996) observed a highly negative correlation of electrolyte leakage rate of cells from leaf blades and heat damage index with heading rate under high temperature. In spring wheat, Xu Ruqiang *et al.* (1997) suggested that membrane thermostability was a better indicator of heat tolerance in the field than susceptibility index. In pearl millet Howarth *et al.* (1997) reported significant correlation between the ability of membrane thermostability to acclimate and seedling survival in the field.

Decreased membrane stability as a result of moisture and heat stress in wheat was reported by Sairam *et al.* (1997). Marcum (1998) identified cellular

membrane thermostability test as an ideal method for screening large numbers of kentucky bluegrass genotypes for heat tolerance, which is rapid and accurate with minimum space requirement. In pepper, changes in cell membrane permeability have been used as criteria for determining the heat tolerance (Yuangan *et al.*, 1998). In *Hevea* Samarappuli and Yogaratnam (1998) described the role of leaf tissue membrane thermostability as an adaptation to drought and global warming.

2.2 Traits associated with drought tolerance

Crop plants adapt to stress conditions by the intervention of several inductive physiological, morphological, biochemical and anatomical mechanisms, which are more or less specific to species (Hanson, 1980; Kramer, 1983). It is important to understand the major mechanisms associated with drought tolerance in order to identify reliable parameters and develop effective screening techniques for screening germplasm accessions as well as progeny from breeding programmes.

2.2.1 Physiological changes induced by water stress

Genetic approach to drought resistance by selecting for yield under stress is a possible but a prolonged and problematic procedure. Recent developments in the understanding of the physiological responses of plants to water stress and their associations with plant productivity allow to embark upon experimental selection programmes that employ physiological selection criteria for drought resistance. This is supported by the recent developments of rapid selection techniques for several physiological components of drought resistance. Studies suggest that no singular drought adaptive trait is predictive of plant response to stress (Nass and Sterling, 1981) and hence multiple physiological selection criteria are required.

2.2.1.1 Stomatal conductance, transpiration rate, leaf water potential and soil water potential

Extensive reviews are available on the controlling effect of stomata in the regulation of the water balance of plants (Hsiao, 1973). A number of environmental factors have been shown to influence stomatal response (Burrows and Milthorpe, 1976). In the literature, the stomatal regulation is described in terms of conductance rather than resistance and low conductance has been considered as an important trait for improving yield under water limited environment. Changes in conductance cause changes in Ψ leaf by altering the rate of transpiration. In orange trees, leaves that had undergone severe water stress had lower leaf water potentials, for a given relative water content, than unstressed leaves (Feres *et al.*, 1979). Effect of leaf water potential on the fruit quality of satsuma has been reported by Maotani and Machida (1980) and the effect of soil water potential on cocoa tree growth has been studied by Machado and Alvim (1981).

When young rubber trees were subjected to water stress, the net photosynthesis and stomatal conductance showed a sigmoid shaped declining curve as a function of increasing water stress situation (Ceulemans *et al.*, 1983). The increasing soil dehydration reduced the leaf water potential, transpiration and photosynthesis and increased the stomatal resistance in young *Hevea* (Conceicao, 1985). Effect of water stress on transpiration, photosynthesis, leaf water potential and stomatal conductance has been extensively studied in various fruit-crops such as apple (Lankes, 1985), *Olea europaea* (Jorba *et al.*, 1985), almond, peach and plum (Dettori, 1985). Bannister (1986) observed that the maximum water potentials observed in wilted shoots of some trees were highly correlated with their drought resistance, with the most sensitive species showing wilting at the highest water potentials and the most resistant at the lowest. Studies conducted in olive (Tombesi *et al.*, 1986) and peach trees (Garnier and Berger, 1987) revealed

a negative influence of water stress on photosynthesis, leaf water potential and stomatal conductance. Rajagopal *et al.* (1988) have suggested that leaf water potential measurement can serve as a rapid method of screening for drought tolerance in coconut plantations. In coconut the positive relation between seasonal and daytime fluctuations in leaf diffusive resistance and drought resistance also have been well established (Bai *et al.*, 1988). Influence of soil moisture status during dry and wet periods on yield, yield components and water relations was studied in *Hevea* clones by Devakumar *et al.* (1988). The results indicated that low transpiration coefficients are associated with high yields and drought tolerance in clones RRII 105 and Gl 1. Similar response of *Hevea* clones to water stress has also been confirmed by Rao *et al.* (1988).

Decreased transpiration under water stress was noticed in cocoa accessions (Balasimha and Rajagopal, 1988). Similarly increased stomatal resistance and reduced transpiration rate and leaf water potential were noticed in water stressed coconut palms (Rajagopal *et al.*, 1989). In evergreen sclerophylls leaf water potential and solute water potential were low during drought period (Rhizopoulou and Mitrakos, 1990). Chandrashekar *et al.* (1990) observed that in *Hevea* clones grown in the non-traditional region the extreme soil and atmospheric moisture deficits resulted in very low plant moisture status and high plugging indices and the stomatal conductance and transpiration rates were also severely inhibited throughout the day. While comparing the responses of two *Hevea* clones RRII 105 and RRII 118 to soil moisture status, Rao *et al.* (1990a) observed that the clone RRII 105 was more tolerant of drought due to higher stomatal resistance, higher leaf water potential and lower transpirational water loss. Similar observations were noticed in cocoa by Balasimha *et al.* (1991), in coconut by Shivashankar *et al.* (1991) and in *Hevea* by Mohan Krishna *et al.* (1991). Shivashankar *et al.* (1993) used leaf water potential and stomatal

resistance measurements for comparing the drought tolerance of the hybrid progenies resultant of three cross combinations in coconut.

Reduction in the rate of transpiration was associated with reduction in predawn leaf water potential in *Prunus persica* trees under soil drought (Tavares *et al.*, 1994). Chandrashekar (1997) used the parameters soil moisture, leaf water potential and stomatal conductance, in order to study the performance of certain *Hevea* clones exposed to atmospheric and soil moisture stress under subhumid climatic conditions. Repellin *et al.* (1997) suggested that leaf water status might be useful as early selection criteria for drought resistance in coconut. Valancogne *et al.* (1997) identified predawn leaf water potential as a water stress indicator for irrigation scheduling and for irrigation trials while conducting experiment in different fruit tree species. Significantly higher stomatal conductance to water vapour was noticed in well watered ponderosa pine seedlings by Zhang *et al.* (1997). Vijayakumar *et al.* (1998) observed the indirect effect of stomatal resistance on photosynthesis while studying the irrigation requirement of rubber trees in the subhumid tropics. Low values of stomatal resistance were recorded in plants under higher water regimes.

2.2.1.2 Chlorophyll fluorescence

Chlorophyll fluorescence serves as an intrinsic indicator of the photosynthetic reactions in the chloroplasts of green plants. Studies revealed that chlorophyll fluorescence analysis is a sensitive indicator of stress induced limitations of photosynthesis. High temperature treatment causes a variety of fluorescence changes: at elevated temperatures the dark fluorescence level, F_0 , is increased several-fold (Schreiber and Berry, 1977). Following heat treatment, there is a decrease in variable fluorescence, F_v , as measured upon illumination at room temperature (Santarius and Muller, 1979).

In cocoa accessions, Balasimha (1992) noticed a decrease in the F_v values during the drier months as compared to other months. The F_0 was significantly higher in susceptible accessions showing that PS II was affected to a greater extent and F_m and F_v values were lower in them. In *Nicotiana tabacum*, Eggenberg *et al.* (1995) observed that the total variable fluorescence ($F_m - F_0$) of the resistant cultivars were greater than those of the susceptible cultivars. Upon rewatering, fluorescence signals showed a reverse trend back to normal, appreciably faster in the resistant cultivars than in susceptible cultivars.

Chlorophyll fluorescence parameters as predictive test of drought tolerance have been used in wheat by Al-Hakimi *et al.* (1995). The fluorescence parameter which differed the most in its response to drought stress between the drought resistant and drought susceptible *Nicotiana tabacum* cultivars was F_0 which increased substantially in drought susceptible cultivars (Reusburg *et al.*, 1996). Maury *et al.* (1996) considered F_v/F_m values for assessing the photochemical responses of two sunflower genotypes to drought acclimation. Chlorophyll fluorescence analysis of drought stressed plants of transgenic tobacco showed a higher photochemical quenching and a higher ratio of variable fluorescence over maximal fluorescence (F_v/F_m) indicating a more efficient photosynthesis (Pilon-Smits *et al.*, 1998). Chlorophyll fluorescence was used as a selection criterion for grain yield in *durum* wheat by Araus *et al.* (1998) and observed that in the driest environment the mean values of F_v/F_m and F_m were decreased and F_0 was increased.

2.2.2 Morphological changes induced by water stress

2.2.2.1 Changes related to leaf and stem

Extension growth is generally a more sensitive process than carbon dioxide assimilation during water stress (Boyer, 1970; Hsiao, 1973). Along with this a reduction in leaf area, increased rates of senescence of older leaves,

premature abscission, leaf rolling and folding which in turn reduce the transpiration, occur as a result of water deficit condition. Streitberg (1975) noticed enhanced leaf formation and increased individual and total leaf areas in apple trees grown under higher irrigation levels and reduced light intensity. A reduced shoot extension rate and shrinking of shoots in the drought affected apple trees were reported by Powell (1976).

In coconut, intensity of drought has been assessed by the drought tolerance index (Pomier and de Taffin, 1982) or by the aridity index (Rao, 1985) based on the reduction in the number of leaves and nuts during drought situations. Joly and Hahn (1989) observed a reduction in leaf area in cocoa plants as an adaptive mechanism to circumvent the periods of drought. In *Hevea* clones, Chandrashekar *et al.* (1990) noticed partial defoliation and leaf margin drying during the summer periods in the non-traditional rubber growing area of North Konkan region. In coconut, the number of drooping leaves was higher in drought susceptible genotypes under water stress condition (Rajagopal *et al.*, 1990). Greatest tree height, shoot growth, tree spread and trunk girth were obtained, when apple trees were grown under low moisture stress (Chandal and Chauhan, 1990).

Leaf productivity in white clover cultivars was greatly reduced by moisture stress (Barbour *et al.*, 1995), while plant height in maize was appreciably reduced by drought (Terrazas *et al.*, 1995). A reduction in leaf area and increase in number of dry leaves were reported during dry season in wheat genotypes (Cortazar *et al.*, 1995). In apple trees Yang-Sang Jin *et al.* (1996) noticed decrease in shoot length and leaf area and increase in fruit drop and leaf fall with increasing water stress. Water deficit treatments significantly reduced plant height, leaf area, number of leaves and number of branches in eggplant cultivars (Byari and Al-Rabighi, 1996). A reduction in leaf area was noticed in different cultivars of *Brassica* under water stress (Paelik *et al.*, 1996) and in tef,

drought reduced the leaf area through a reduction in leaf size (Shiferaw and Baker, 1996) and a reduction in plant height and biomass were also observed. In a drought resistant almond cultivar, Herralde *et al.* (1997) noticed larger leaves and a more open and denser crown. Leaf rolling was noticed where rice and wild *Oryza* species were grown under water limiting growth conditions (Yeo *et al.*, 1997). Moisture deficit resulted in a reduction in plant height and number of tillers in tef (Takele, 1997). In *Hevea* Vijayakumar *et al.* (1998) noticed elimination of foliar injury under sufficient irrigation and Devakumar *et al.* (1999) reported changes in canopy architecture as a result of drought.

2.2.2.2 Root, root:shoot ratio and dry matter production

In coconut palms under severe moisture stress, dry matter production was reduced by 22 per cent as compared with well-watered palms (Rajagopal *et al.*, 1989). Fall in plant biomass and yield was reported in drought stressed maize by Celiz *et al.* (1995). Water deficit reduced the fresh and dry weights of leaves, stem and roots in egg plant (Byari and Al-Rabighi, 1996). Kobata *et al.* (1996) reported high dry matter production of the shoot in drought resistant rice cultivars where water consumption was highly correlated with root density in deep soil layers. Response of young tea clones to drought and temperature was studied by Burgers and Carr (1996) who observed that the amount of dry matter partitioned to leaves, stems and harvested shoots declined by 80-95% by drought treatment. Effect of water stress on root response and dry matter production in apple trees has been reported by Trunov (1996) and Fernandez *et al.* (1997).

In field bean and pea, drought treatment resulted in a significant decrease in the number of developed lateral roots, their total length and dry matter (Grzesiak *et al.*, 1997a). In a laboratory study Gareia and Gonzalez (1997) identified length of root as the most appropriate indicator for evaluating water stress tolerance in rice varieties. A lower shoot:root ratio was characteristic of

drought resistant field bean cultivars (Grzesiak *et al.*, 1997b). In cowpea, distribution percentage of dry matter to roots was higher in the tolerant lines with their root weight increasing steadily even at maturity in contrast with a considerable decline in the susceptible ones.

2.2.2.3 Effects on *Hevea* growth and yield

Yield reduction in *Hevea* during drought period has been undoubtedly proved both in the traditional and nontraditional rubber growing areas. Conceicao *et al.* (1986) noticed a reduction in growth and assimilate partitioning when *Hevea* clones were subjected to a water deficit. Devakumar *et al.* (1988) conducted a study to understand the influence of soil moisture status during dry and wet periods on yield components and water relations in four *Hevea* clones. Low dry rubber yield in all the clones was associated with high plugging index and low initial flow rate of latex in the dry season. Rao *et al.* (1988) observed significant variations in yield components between the two *Hevea* clones during water stress period. Similarly Rao *et al.* (1990b) reported significant clonal and seasonal variations in yield, yield components and components of water relations in two *Hevea* clones, RRII 105 and RRII 118.

Seasonal changes in yield was studied in *Hevea* clones by Chandrashekar *et al.* (1990) in the non-traditional rubber growing area of north Konkan region. They observed that the per tap dry rubber yield in summer months was extremely low and uneconomical. In another study on growth reaction of *Hevea brasiliensis* to heat and drought stress under dry subhumid climatic conditions Chandrashekar *et al.* (1996) observed growth only during the monsoon period while in dry period there was a reduction in tree girth. Similarly in an analysis of growth and drought tolerance in rubber during the immature phase in the dry subhumid climate, Chandrashekar *et al.* (1998) noticed that a large portion of growth occurred only in the wet season whereas the growth rates of the clones

during the dry season declined substantially and a decrease in tree girth was noticed in most of the clones. Reduction in *Hevea* growth and yield as a result of drought is also reported by Annamalainathan *et al.* (1998) and Devakumar *et al.* (1998).

2.2.3 Biochemical changes induced by water stress

Electrolyte leakage from cell membranes as a result of heat and drought stress as well as changes in chlorophyll and epicuticular wax contents are among the biochemical parameters considered in the present study. Other biochemical changes like accumulation of osmotically active organic solutes in the free or uncombined form occur when plants are exposed to various environmental stresses.

2.2.3.1 Chlorophyll Content and Chlorophyll Stability index

A high chlorophyll stability and a high correlation between chlorophyll content, apparent photosynthesis and final yield has been reported in coconut by Mathew and Ramadasan (1975). In *Zea mays*, Alberte *et al.* (1977) reported substantial loss of chlorophyll from the mesophyll chloroplast during water stress. In barley, Bhardwaj and Singhal (1981) observed a reduction of chlorophyll a/b protein complex in light under water stress. Chlorophyll stability has been used as an index of drought resistance in sugarcane (Sharma and Gill, 1981).

Shivashankar *et al.* (1991) observed reduction in the total chlorophyll in the unirrigated coconut palms compared to irrigated palms, as an effect of water deficit. Reduction in total chlorophyll content, as a result of water stress is reported in different cultivars of *Brassica napus* by Paelik *et al.* (1996) and in cabbage by Chauhan and Senboku (1996). Moisture stress and temperature stress decreased the chlorophyll content and chlorophyll stability index in wheat

(Sairam *et al.*, 1997), maize (Gutierrez *et al.*, 1998), *Hevea* (Vijayakumar *et al.*, 1998) and in *Brassica carinata* (Voleti *et al.*, 1998).

2.2.3.2 Epicuticular wax content (ECW)

The cuticular wax plays an important role in reducing evaporation from the leaf surface (Jordan *et al.*, 1983a) and in increasing the yield stability in water limited environments (Johnson *et al.*, 1983). Presence of ECW helps in reducing cuticular transpiration (Rao, 1983), stomatal transpiration and promotes reflection of radiant energy by canopies (Lee and Graham, 1986). Conditions favourable for high wax production are high radiant energy, high temperature, low humidity and increased water stress (Svenningsson and Liljenberg, 1986). Rainfall removes ECW from the leaf surfaces (Mayeux and Jordan, 1987). Jefferson *et al.* (1988) observed increased ECW production in drought stressed alfalfa plants and identified this as a potential selection criterion for drought resistance. Higher wax content had been observed during summer than rainy season in rubber (Rao *et al.*, 1988) and in coconut (Kurup, 1989).

In coconut, ECW has been used as an important trait for screening the genotypes for drought resistance. The drought tolerant palms exhibited higher wax content as compared to the susceptible types during peak summer months (Rajagopal *et al.*, 1990). An inverse relationship between ECW content and transpiration rate has been reported in coconut by Rajagopal *et al.* (1991). In *Hevea* clones, Vijayakumar *et al.* (1998) observed increased wax content in leaf surface of rainfed plants compared to irrigated plants.

2.2.4 Anatomical traits associated with drought tolerance

Streitberg (1975) noticed that the number of stomata and proportion of palisade tissue were lower when apple trees were grown under higher irrigation levels. In sorghum, a strong correlation between thick waxy cuticle and drought

resistance has been observed (Blum, 1975). Stomatal frequency, size and responsiveness are the factors, which control the water loss of the plants (Parsons, 1979). In an anatomical comparison of leaves of a diploid and two polyploid clones of *Hevea brasiliensis*, Medri and Lleras (1981) observed that the polyploids are more resistant to drought than the diploid. Stomata play an important role in controlling the balance between assimilation and transpiration (Jordan *et al.*, 1983b). In coconut, varietal differences in the stomatal density has been reported by Rajagopal *et al.* (1990). In a study on comparative bark anatomy of drought tolerant and susceptible *Hevea* clones, Premakumari *et al.* (1993) observed significant differences in the characters such as height and width of phloic rays, height:width ratio of phloic rays, the 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. While evaluating the drought tolerance of pepper cultivars, Lee Woosung *et al.* (1996) observed a significant positive correlation between stomatal density and water saturation deficit value.

Sam *et al.* (1996) reported the presence of thicker mesophyll and spongy parenchyma in tomato and potato cultivars which were more tolerant to water and heat stresses. Similarly higher values of leaf thickness, palisade tissue thickness, palisade tissue to spongy tissue ratio and stomatal density were noted in the drought resistant cultivars of kiwifruit by Pong Yong Hong and Zhang Wen Cai (1996). While studying the effect of drought on the roots of various temperate fruit-crops, Trunov (1996) suggested that the varietal differences noticed in response to drought was partly owing to the structure of its leaf, which favoured economical utilization of moisture. Chowdhary *et al.* (1996) noticed positive significant correlation between stomatal density, leaf venation and grain yield of wheat lines grown under drought conditions. In drought resistant variety of kiwifruit higher density of stomata was reported by Wang Rencai (1997), whereas drought resistant field bean cultivars were characterized by lower frequency and

size of stomata (Grzesiak *et al.*, 1997). In a study on permanent wilting point in some coffee selections, Bayan and Bora (1997) noticed significant influence of stomatal density on the time taken to reach the permanent wilting point. Better stomatal and cuticular control of water loss was noticed in drought resistant hybrid poplar clones (Harvey and Driessche, 1997). Thickness of cuticle, ratio of palisade to spongy tissue, thickness of mesophyll tissue, extent of palisade cell density, diameter of main leaf vein and layers of collenchyma cells each side of the main vein were significantly different in the drought resistant varieties of *Juglans regia* and *Juglans sigillata* (Mei Xiuying *et al.*, 1998). Higher stomatal density in the dwarf varieties of coconut is the reason for their drought susceptibility according to Juma *et al.* (1998).

2.3 Genotypic differences in drought tolerance

There are several reports to show the genotypic variation for each of the above mentioned drought related characters. The greatest potential in breeding and selection for adaptation to drought seems to lie in specific processes controlled by one or a few genes, such as waxy layer on leaves or osmoregulation, rather than integrated traits controlled by many genes (Morgen, 1988). Hence, selection at genotypic level offers possibility to evolve suitable drought tolerant varieties. Rao *et al.* (1988) observed highly significant clonal variations in the levels of ECW in the young rubber plants studied. Clonal variations in response to drought in terms of yield and associated physiological parameters in *Hevea* clones were reported by Vijayakumar *et al.* (1988).

Genotypic differences for chlorophyll content, stomatal resistance and individual leaf area were reported in *Brassica* by Hobbs (1988). Devakumar *et al.* (1988) suggested that maintenance of higher stomatal resistance inspite of better water status in *Hevea* clone RRII 105 might be an indication of genetic variation of this clone in stomatal response to leaf water potential. Significant genotypic

differences for ECW content are reported in cocoa by Balasimha *et al.* (1988) and in coconut accessions by Rajagopal *et al.* (1990). Significant genotypic differences for various drought related characters were noticed in several crops like *Hevea* (Nazeer *et al.*, 1992), tea (Satyanarayana and Spurgeon Cox, 1994), wheat (Kumar *et al.*, 1995), sunflower (Reddy *et al.*, 1995), onion (Pathak *et al.*, 1996), pine seedlings (Tognetti *et al.*, 1997) and in tomato (Rahman *et al.*, 1998).

Materials and Methods

MATERIALS AND METHODS

Wild germplasm conserved in the source bush nursery of Central Experimental Station (CES) of Rubber Research Institute of India (RRII) constituted the base material for the study. Based on a preliminary observation on growth parameters, 450 accessions were selected and planted in the field of CES during 1990 for detailed studies. Out of these 450 accessions 250 were selected based on juvenile growth and vigour and observations were taken for various characters. On the basis of this study and with special reference to stem girth increment during the summer period, these 250 accessions were classified using growth index data. Out of these 250 accessions, 99 accessions belonging to Acre and Mato Grosso states of Brazil were selected. The selection was in such a way as to include the maximum variability among genotypes for summer girth. The 99 accessions selected (Table 1), constitute the materials for the present investigation.

The entire study was conducted in three experimental stages at RRII. The results obtained in the first experimental stage were utilised for the ensuing experiment in the second stage. The methodology followed in each experiment is presented below.

3.1 Experiment I - Preliminary screening of wild *Hevea* germplasm for drought resistance based on cell membrane stability

Ninety nine genotypes of the Brazilian germplasm planted during 1990 at the Central Experimental Station of the Rubber Research Institute of India, Kottayam were selected for the study during 1998, along with the standard clone RRII 105. These genotypes were representative of Acre and Mato Grosso states of Brazil. The plants were planted in 2 m spacing and three trees per genotype were selected.

Table 1. List of 99 wild genotypes of *Hevea brasiliensis* selected for the study

Sl. No.	National accession number	International genotype code	Sl. No.	National accession number	International genotype code	Sl. No.	National accession number	International genotype code	Sl. No.	National accession number	International genotype code
1	AC 175	AC/T/1-5/149	26	MT 182	MT/C/2-10/8	51	AC 643	AC/S/11-41/281	76	AC 606	AC/S/11-41/11
2	AC 446	AC/F/5-21/208	27	MT 142	MT/PB/1-2/33	52	AC 716	AC/S/12-42/173	77	AC 658	AC/S/9-39/16
3	AC 163	AC/T/1-5/74	28	MT 186	MT/C/2-10/18	53	AC 661	AC/S/9-39/22	78	MT 927	MT/IT/16-34/132
4	AC 168	AC/T/1-5/120	29	MT 194	MT/C/2-10/33	54	AC 788	AC/F/6A-36/199	79	MT 38	MT/IT/40-30/6
5	AC 165	AC/T/1-5/90	30	MT 191	MT/C/2-10/29	55	AC 640	AC/F/11-41/260	80	MT 64	MT/IT/40-30/85
6	AC 177	AC/T/1-5/166	31	MT 57	MT/17/14-30/53	56	AC 710	AC/S/12-42/67	81	MT 40	MT/IT/40-30/9
7	AC 155	AC/T/1-5/26	32	MT 63	MT/17/14-30/84	57	AC 749	AC/S/10-37/64	82	MT 45	MT/IT/40-30/18
8	AC 160	AC/T/1-5/55	33	MT 69	MT/17/14-30/105	58	AC 633	AC/S/11-41/212	83	MT 58	MT/IT/40-30/67
9	AC 162	AC/T/1-5/70	34	MT 56	MT/17/14-30/42	59	AC 708	AC/S/12-42/56	84	MT 945	MT/IT/16-34/210
10	AC 166	AC/T/1-5/109	35	MT 202	MT/C/2-10/57	60	AC 713	AC/S/12-42/120	85	MT 924	MT/IT/16-34/119
11	AC 161	AC/T/1-5/60	36	MT 67	MT/17/14-30/95	61	AC 775	AC/S/10-37/131	86	MT 913	MT/IT/16-34/49
12	MT 197	MT/C/2-10/39	37	MT 44	MT/17/14-30/15	62	AC 795	AC/F/6A-36/229	87	MT 904	MT/IT/16-34/9
13	MT 198	MT/C/2-10/41	38	MT 73	MT/17/14-30/121	63	AC 635	AC/S/11-41/240	88	MT 930	MT/IT/16-34/137
14	MT 196	MT/C/2-10/36	39	MT 43	MT/17/14-30/14	64	AC 723	AC/S/12-42/258	89	MT 919	MT/IT/16-34/79
15	MT 199	MT/C/2-10/51	40	MT 78	MT/17/14-30/134	65	AC 756	AC/S/10-37/81	90	MT 943	MT/IT/16-34/203
16	MT 188	MT/C/2-10/21	41	MT 68	MT/17/14-30/100	66	AC 621	AC/S/11-41/90	91	MT 942	MT/IT/16-34/202
17	MT 187	MT/C/2-10/19	42	MT 80	MT/17/14-30/137	67	AC 761	AC/S/10-37/86	92	MT 66	MT/IT/14-30/93
18	MT 189	MT/C/2-10/22	43	AC 762	AC/S/10-37/87	68	AC 760	AC/S/10-37/85	93	MT 55	MT/IT/14-30/39
19	MT 185	MT/C/2-10/16	44	AC 676	AC/S/9-39/67	69	AC 688	AC/S/9-39/116	94	MT 76	MT/IT/14-30/131
20	MT 193	MT/C/2-10/32	45	AC 697	AC/S/9-39/169	70	AC 700	AC/S/9-39/180	95	MT 41	MT/IT/14-30/12
21	MT 184	MT/C/2-10/13	46	AC 750	AC/S/10-37/65	71	AC 648	AC/S/11-41/342	96	AC 652	AC/S/11-41/364
22	MT 180	MT/C/2-10/5	47	AC 727	AC/S/10-37/3	72	AC 1044	AC/S/12-42/366	97	AC 728	AC/S/10-37/7
23	MT 179	MT/C/2-10/4	48	AC 769	AC/S/10-37/118	73	AC 702	AC/S/12-42/12	98	AC 650	AC/S/11-41/348
24	MT 178	MT/C/2-10/3	49	AC 765	AC/S/10-37/101	74	AC 631	AC/S/11-41/194	99	MT 938	MT/IT/16-34/184
25	MT 181	MT/C/2-10/6	50	AC 729	AC/S/10-37/8	75	AC 537	AC/F/6A-36/68			

The method used for measuring cell membrane stability of leaves of *Hevea brasiliensis* was that of Sullivan (1972). Fully expanded leaves of physiologically similar stage of maturity were collected and washed in deionised water before punching the middle leaflets. Each sample for assay consisted of a paired set, control (C) and treatment (T) of 20 leaf disc samples cut from a group of 20 leaflets with a 1 cm diameter specially constructed leaf disc punch.

Prior to assay, the paired set of leaf discs was placed in two separate test tubes and washed thoroughly in distilled water, with at least four changes of water to remove exogenous electrolytes adhering to tissue surfaces and endogenous electrolytes released from cut cells at the periphery of the discs. The sample without any treatment served as control. The following procedure was carried out.

The "treatment tubes" were first incubated in 20 ml polyethylene glycol (PEG 6000) solution (60%) for 24 hrs at 10°C, washed repeatedly with distilled water and kept in a controlled water bath at 45°C for one hour. The volume was made upto 30 ml and the solution was incubated at 10°C for 18 hours for the diffusion of electrolytes. The sample tubes were brought to room temperature and the initial conductance was read using a "Systronics 305" conductivity bridge. On completion of the initial reading, the control and treatment tubes were autoclaved at 1.4 kg/cm² for 15 minutes to kill the leaf tissue completely. The tubes were then brought to room temperature, the contents mixed thoroughly and the final conductance was read. The degree of injury to the cell membrane was calculated as follows.

$$\% \text{ injury} = \left[1 - \frac{(1-T_1/T_2)}{(1-C_1/C_2)} \right] \times 100$$

where, T_1 and T_2 are the initial and final conductance of treatments, and C_1 and C_2 are the initial and final conductance of controls respectively. T_1/T_2 , the ratio of initial conductance to final conductance is a relative measure of the amount of electrolyte leakage induced by the treatments and is assumed to be proportional to the amount of injury induced in cell membranes.

Based on this study, these 99 genotypes were classified into five groups and 10 genotypes, two from each group were selected for the detailed study in the second experiment. The wild genotypes selected were AC 1044, MT 55, AC 446, MT 41, MT 76, MT 66, MT 938, AC 650, AC 652 and AC 728.

3.2 Experiment II - Extent of genetic variability among the selected genotypes for drought tolerance using various indices (Field experiment)

The 10 genotypes selected from Experiment I were multiplied at RRII along with the control clones RRII 105 (popular high yielding clone), RRIM 600 (drought tolerant clone) and Tjir1 (drought susceptible clone) as budded stumps during 1999. They were grown in polythene bags of lay flat dimension 55 cm x 25 cm and 400 gauge thickness. The budded stumps planted in the polythene bags were irrigated well till they were established. During the month of March, 2000 when the polybag plants were 10 months old, the entire polybag plants were divided into two sets - one control set where the irrigation was continued on alternate days and the other treatment set where the irrigation was stopped.

3.2.1 Physiological parameters recorded

Certain drought related physiological parameters were recorded from the stressed plants during the following levels of water stress

1. Control (NS)
2. Water stress for 15 days (S-1)

3. Water stress for 30 days (S-2)
4. Water stress for 45 days (S-3)

Corresponding observations from the control set plants were also recorded. After withholding irrigation for 45 days, the plants in the treatment set were given two days irrigation and the observations were recorded after a week to know the post stress (PS) effect.

Several physiological parameters were measured from the middle leaflet of the middle leaves of the youngest fully mature flush in each plant. The parameters recorded were transpiration (E), stomatal resistance (r_s), leaf temperature ($^{\circ}\text{C}$), chlorophyll fluorescence and afternoon leaf water potential (Ψ_{leaf}). Stomatal conductance (g_s) was worked out using stomatal resistance recordings. Afternoon soil water potential (Ψ_{soil}) was also recorded corresponding to the water stress levels. Stomatal resistance, transpiration rate and leaf temperature were measured during 08.30 – 10.30 h using LI – 1600 Steady State Porometer (Licor Instruments, USA). Chlorophyll fluorescence was recorded during 11.30 – 12.30 h using Plant Efficiency Analyzer (Hansatech Instruments Limited, England). Leaf water potential and soil water potential were recorded during 14.00 – 15.00 h using C – 52 sample chamber psychrometer (Wescor Inc., Logan, USA) connected to HR 33T Dew Point Microvoltmeter.

3.2.2 Morphological parameters recorded

To know the genotypic differences among the 10 selected genotypes for growth and vigour, the following morphological observations were recorded from the plants under irrigation at the age of 10 months.

1. Scion height (cm)
2. Scion basal diameter at 20 cm from bud union (mm)
3. Number of leaves

4. Number of leaf flushes
5. Interflush distance (cm)
6. Single leaflet area (cm²)
7. Specific leaf weight (SLW) (g.cm⁻²)

Leaflet area was recorded using LI-3100 area meter (Licor Instruments, USA).

For recording SLW area of middle leaflets was recorded and they were oven dried at 80°C for two days. The following formula is applied.

$$\text{SLW (g.cm}^{-2}\text{)} = \frac{\text{Leaf dry weight}}{\text{Leaf area}}$$

3.2.3 Biochemical parameters recorded

The following biochemical parameters were recorded from the plants under irrigation.

1. Total chlorophyll content (mg cm⁻²)
2. Chlorophyll reduction percentage
3. Epicuticular wax content (µg cm⁻²)

3.2.3.1 Total chlorophyll content

Total chlorophyll was estimated by the method of Ozerol and Titus (1965).

Fresh leaves at physiologically similar stage of development were collected for the estimation of total chlorophyll. Twenty discs were punched from leaves of each plant and their total area was recorded. The discs were soaked in 10 ml of methanol in small vials and were kept in dark for 24 hrs. Optical density

of the methanol extract was measured at 651 and 664 nm in a UV Spectrophotometer. Pure methanol was taken as blank. The total chlorophyll was calculated from the following equation.

$$C \text{ (mg cm}^{-2}\text{)} = 25.5 D_{651} + 4.0 D_{664} \text{ mg l}^{-1} \text{ of chlorophyll in methanol}$$

where

C = Total chlorophyll (mg cm⁻²)

D₆₅₁ = Optical density of the extract at 651 nm

D₆₅₄ = Optical density of the extract at 664 nm

3.2.3.2 Chlorophyll reduction percentage

This method suggested by Dhopte and Livera (1989) is based on pigment changes induced by heating. The chlorophyll destruction commences rapidly at critical temperature of 55-56°C. Thus, chlorophyll stability is a function of temperature.

The leaf samples were collected in two sets. One set was placed in test tubes containing 50 ml of distilled water and these tubes were kept in a hot water bath maintained at 56 ± 1°C for exactly 30 minutes. Another set was kept at room temperature to serve as control. The total chlorophyll content was estimated from both the sets separately using the method of Ozerol and Titus (1965).

From this chlorophyll reduction (%) was worked out as follows:

$$\text{Chlorophyll reduction (\%)} = \frac{\text{Total chlorophyll content in control} - \text{Total chlorophyll content after heating at } 56^{\circ}\text{C}}{\text{Total chlorophyll content in control}} \times 100$$

3.2.3.3 Epicuticular wax content

The wax content was determined by the spectrophotometric method of Ebercon *et al.* (1977). This method is based on the colour change produced by the reaction of wax with acidic potassium dichromate ($K_2Cr_2O_7$).

Ten leaf discs of 1 cm^2 area from physiologically mature leaves of the top flush were immersed in 15 ml of chloroform for 15 seconds. The extract was filtered and evaporated to dryness on boiling water bath, until the smell of chloroform was fully vanished. Five ml of acidic $K_2Cr_2O_7$ were added to the samples placed in boiling water bath for 30 minutes. After cooling, 12 ml of deionised water was added. Fifteen minutes were allowed for the colour development and cooling after which the optical density of the samples was read at 590 nm in a UV spectrophotometer and expressed in $\mu\text{g. cm}^{-2}$ using a standard curve.

3.2.4 Anatomical parameters recorded

3.2.4.1 Leaf anatomical characters

3.2.4.1.1 Observation through leaf cross section

The following measurements were recorded from the cross section of the leaf of the non-stressed plants.

1. Thickness of palisade tissue (μm)
2. Thickness of mesophyll tissue (μm)
3. Mean number of cells in unit length of palisade layer
4. Leaf thickness (μm)
5. Leaf vein (midrib) diameter (μm)

3.2.4.1.2 Stomatal density

Epidermal peelings were separated by boiling the leaf bits in 60 per cent Nitric acid with a pinch of potassium chlorate. The peelings were thoroughly cleaned, stained with Safranin and observed under a light microscope. The stomatal count of the lower epidermis was expressed as number of stomata per square mm.

3.2.4.2 Bark anatomical characters

Bark samples were collected from 16 months old plants at a height of 15 cm from the bud union. Sections were cut using a sledge microtome and the following characters were recorded.

1. Total number of latex vessel rows (L V R)
2. Number of L V R in the soft bast
3. Total bark thickness (mm)
4. Thickness of soft bast (mm)

3.3 Experiment III – Extent of genetic variability among the selected genotypes for certain drought related morphological indices (Glass house experiment)

In order to avoid the influence of untimely rains, which occurred during the recording period a complete set of the selected genotypes were grown in polybags inside the glass house. After the establishment of the plants, they were divided into two sets. In one set irrigation was given on alternate days, and in the second set of plants irrigation was withheld for 60 days. At the end of two months stress period the following morphological observations were recorded.

1. Basal diameter of scion (mm) - before and after inducing stress in both sets
2. Fresh weight of the scion (g) } From the stressed and non stressed plants after two months period
3. Dry weight of the scion (g) }

Dry matter stress tolerance index (DMSI) was worked as follows:

$$\text{DMSI} = \frac{\text{Dry matter of non stressed plants} - \text{Dry matter of stressed plants}}{\text{Dry matter of non stressed plants}} \times 100$$

3.4 Statistical analysis

Analysis of variance was done as per Completely Randomised Design (CRD) and Factorial CRD. Based on the significance of F value, genotypes were ranked following Duncan's multiple range test. Other genetic parameters namely, genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), broad sense heritability (h^2) and genetic advance (GA) were estimated using the following formulae. (Allard, 1960; Singh and Chowdhary, 1985).

$$\text{GCV} = \frac{G^2}{\text{General mean}} \times 100$$

where G^2 is the genotypic variance

$$\text{PCV} = \frac{P^2}{\text{General mean}} \times 100$$

where, P^2 is the phenotypic variance

$$h^2 = \frac{G^2}{P^2} \times 100$$

$$\text{GA} = P^2 \times 2.06 \times h^2$$

where 2.06 is the selection differential

$$G^2 = \frac{\text{MSS}_t - \text{MSS}_E}{r}$$

where MSS_t is the treatment mean sum of square
 MSS_E is the error mean sum of square
 r is the replication

$$P^2 = G^2 + MSS_E$$

Genotypic and phenotypic correlations (Singh and Chowdhary, 1985) were worked out to understand the nature and degree of the relationship among physiological, morphological, biochemical and anatomical parameters studied. Cluster analysis (D^2) (Singh and Chowdhary, 1985) was done to assess the genetic divergence among the 10 selected genotypes and group them into different clusters based on the genetic distance. Individual performance of the genotypes was assessed by summing up of the rank values obtained for each character under selection, based on the parametric relationship of these characters to drought tolerance.

Results

RESULTS

The results are furnished below under the following heads :

1. Genetic variability for cell membrane stability among wild *Hevea* germplasm
2. Genetic variability for drought related physiological parameters
3. Genetic variability for drought related morphological parameters
4. Genetic variability for drought related biochemical parameters
5. Genetic variability for drought related anatomical parameters
6. Genetic parameters for selected characters
7. Character association among selected characters
8. Genetic divergence among the selected genotypes for the characters studied
9. Identification of superior genotypes based on rank sum obtained for characters under selection

4.1 Genetic variability for cell membrane stability among wild *Hevea* germplasm

The extent of variability among the wild *Hevea* germplasm for cell membrane stability as indicated by relative injury to cell membrane is given in Table 2. The range varied from 30 – 80 per cent among the accessions with a mean of 53 per cent. The genotypes AC 446, AC 652, MT 80 and AC 643 showed the highest tolerance to water and temperature stresses as indicated by relative injury. The reference clone RRII 105 showed 53.94 per cent relative injury indicating moderate tolerance towards water and heat stresses. The relative injury to cell membrane was highest for the genotypes AC 728, MT 58, MT 927 and AC 650.

The genotypes were classified by ranking them using general mean and SD values (Fig.1). Genotypes with values above mean +SD (>65.23) could be considered to be very susceptible to drought and were ranked as very low

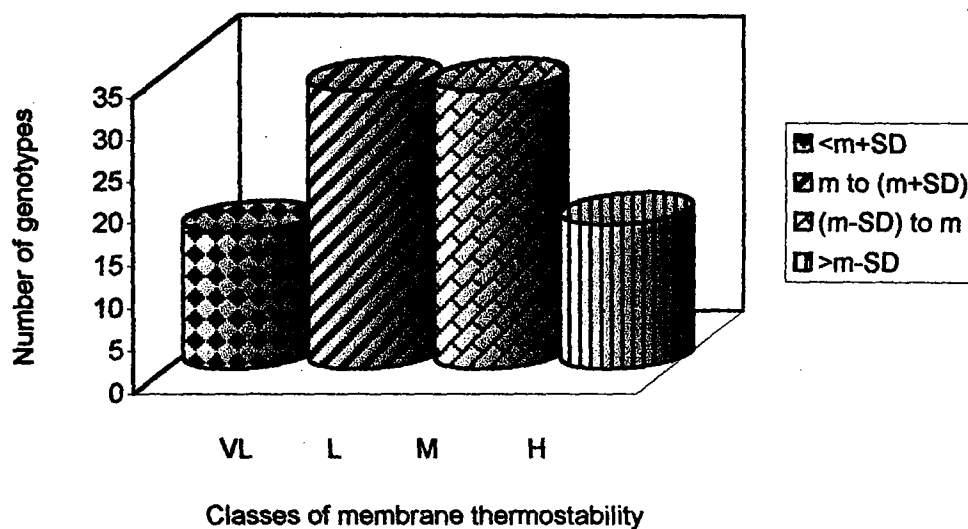
Table 2. Genotypic variation for cell membrane injury among wild *Hevea* germplasm

Genotype	% injury	Genotype	% injury	Genotype	% injury	Genotype	% injury	Genotype	% injury
AC 446	29.77	AC 750	45.06	MT 186	53.69	MT 943	60.32		
AC 652	29.86	AC 640	45.67	MT 198	53.8	AC 155	60.41		
MT 80	29.96	MT 68	46.26	AC 749	53.85	MT 193	61.3		
AC 643	33.03	MT 945	46.45	RRII 105	53.94	MT 930	62.22		
AC 175	33.05	MT 181	46.5	MT 43	53.96	MT 189	62.28		
MT 45	35.11	MT 919	46.95	MT 78	53.99	AC 635	62.45		
AC 537	35.68	MT 924	47.58	AC 166	54.11	AC 676	62.62		
MT 182	35.68	MT 180	47.8	AC 606	54.12	MT 199	63.14		
MT 64	38.5	MT 179	48.01	MT 191	55.49	MT 57	65.12		
MT 40	39.59	MT 184	48.17	MT 202	55.57	AC 760	65.55		
AC 727	40.08	MT 67	48.69	MT 178	56.32	AC 631	66.7		
AC 648	40.34	MT 913	48.74	MT 938	56.34	MT 69	67.35		
AC 661	40.37	AC 716	49.41	MT 904	56.67	AC 756	68.9		
AC 788	40.43	MT 187	49.73	AC 1044	56.82	AC 621	69.51		
AC 161	40.75	MT 142	49.75	AC 702	56.89	AC 775	69.84		
AC 168	41.04	AC 688	50.23	AC 165	57.64	AC 710	69.96		
AC 658	41.24	AC 729	50.28	AC 177	57.93	AC 633	70.06		
AC 765	42.03	MT 41	50.65	AC 697	58.05	AC 723	70.27		
MT 44	43.26	AC 160	51.12	MT 185	58.33	AC 761	71.45		
MT 196	43.3	MT 76	51.29	MT 188	58.53	AC 650	75.93		
AC 762	43.88	AC 162	52.43	AC 700	58.62	MT 927	77.05		
MT 55	44.23	AC 163	52.49	MT 194	58.87	AC 713	77.16		
MT 942	44.58	MT 63	52.49	MT 73	59.58	AC 795	78.97		
MT 197	44.64	MT 38	52.76	MT 56	59.67	MT 58	80.29		
MT 66	45.04	AC 769	53.12	AC 708	60.25	AC 728	80.43		

General Mean = 53.39 CD (0.05) = 3.56

Variance Ratio = 220.01** ** - Significant at P = 0.01

Fig. 1. Variability in membrane thermostability of leaf tissues among wild *Hevea* germplasm collection



m = Mean

SD = Standard deviation

VL = Very low

L = Low

M = Medium

H = High

Table 3. Phenotypic and genotypic coefficients of variation (PCV, GCV), Heritability (H^2) and genetic advance (GA) for cell membrane thermostability

GCV	PCV	H^2	GA
21.54	23.09	87	22.2

performers (VL), with respect to this character. Those with values between mean and mean + SD (53.39 – 65.23) could be considered to be susceptible to drought and were ranked as low performers (L). Genotypes with values between mean – SD and mean (41.55 – 53.39) could be considered to be moderately susceptible to drought and were ranked as medium performers (M) whereas genotypes with values less than mean – SD (41.55) could be considered to be resistant to drought and were ranked as high performers (H). Based on the above classification, 16 genotypes are ranked as very low performers, 33 each as low and medium performers and 17 genotypes as high performers. This method of classification makes the selection procedure more easy while going for further crop improvement programme.

Analysis of data indicated significant genotypic difference for this character among the wild *Hevea* germplasm. Table 3 gives the split up of the total variance into heritable and non-heritable components and also the heritability estimates in the broad sense and genetic advance as percentage of mean. Cell membrane stability indicated a moderate genotypic coefficient of variability (GCV) of 21.54 per cent with a high heritability (87%) and a moderate genetic advance of 22.2 per cent.

On the basis of this study, the genotypes AC 652, AC 446 (H), MT 55, MT 66, MT 41(M), MT 76, MT 938, AC 1044 (L), AC 650 and AC 728 (VL) representative of all range of classification were selected for the second experiment in order to include maximum genetic variability among the selected genotypes.

4.2 Genetic variability for drought related physiological parameters among the selected genotypes.

For comparing the performance of selected genotypes under various water stress levels, the induced water stress levels are hereafter referred to as non-

stress (NS), mild stress (S-1), medium stress (S-2), severe stress (S-3) which were recorded at 15 days interval and the performance at post stress level is designated as (PS). The corresponding periods in the control are designated as (NS), (NS-1), (NS-2), (NS-3) and (NS-4).

4.2.1 Leaf temperature

The mean leaf temperature of *Hevea* genotypes under various levels of induced water stress is shown in Table 4. During the non-stress period the mean leaf temperature was 34.53°C, ranging from 33.69°C to 35.61°C, whereas in stress (S-2) period, the mean leaf temperature was 33.36°C with a range of 30.51°C to 34.39°C. When the leaf temperature was recorded at stress (S-3) period, the mean leaf temperature was increased to 34.9°C with a range of 34.43°C to 35.71°C. During the post stress period the mean leaf temperature was reduced to 34.08°C ranging from 33.34°C to 34.71°C.

Data were analysed separately for each water level and no significant genotypic difference was noticed for leaf temperature under non-stress and stress (S-2) water levels. The clone Tjir1 recorded the minimum leaf temperature under non-stress condition, whereas MT 55 recorded the maximum. At water stress level S-2, the minimum temperature was recorded by AC 728 and the maximum by RRII 105. When the leaf temperature was recorded after a period of 45 days of water stress (S-3), there was significant genotypic difference. The clone Tjir1 recorded the minimum leaf temperature and the genotype MT 76 recorded the maximum leaf temperature. After 2 days irrigation, when the leaf temperature was recorded at post-stress level, the standard clone RRII 105 recorded the minimum temperature and the maximum was recorded by MT 76, but the genotypic difference was not significant. The effect of various water stress levels on leaf temperature is represented in Fig.2.

Table 4. Leaf temperature (°C) of selected genotypes of *Hevea brasiliensis* at varying levels of water stress

Genotype	Non-stress (NS)	Stress (S-2)	Stress (S-3)	Post stress (PS)
AC 1044	33.89 ^b	33.39 ^{ab}	34.54 ^b	34.69 ^{ab}
MT 55	35.61 ^a	33.21 ^{ab}	34.69 ^b	34.09 ^{abc}
AC 446	34.71 ^{ab}	33.31 ^{ab}	35.08 ^{ab}	34.14 ^{abc}
RRIM 600	34.06 ^b	33.50 ^{ab}	34.71 ^b	33.72 ^{abc}
Tjir1	33.69 ^b	33.27 ^{ab}	34.43 ^b	34.01 ^{abc}
MT 41	34.48 ^{ab}	33.10 ^{ab}	35.56 ^a	34.37 ^{abc}
MT 76	34.84 ^{ab}	33.06 ^{ab}	35.71 ^a	34.71 ^a
MT 66	34.52 ^{ab}	33.86 ^a	34.78 ^b	34.30 ^{abc}
MT 938	34.93 ^{ab}	33.72 ^a	34.48 ^b	34.28 ^{abc}
AC 650	33.93 ^b	34.06 ^a	35.00 ^{ab}	34.16 ^{abc}
AC 652	34.53 ^{ab}	34.34 ^a	35.11 ^{ab}	33.60 ^{bc}
RRII 105	34.72 ^{ab}	34.39 ^a	35.01 ^{ab}	33.34 ^c
AC 728	34.93 ^{ab}	30.51 ^b	34.61 ^b	33.66 ^{abc}
Mean	34.53	33.36	34.90	34.08

Any two means having a common letter are not significantly different

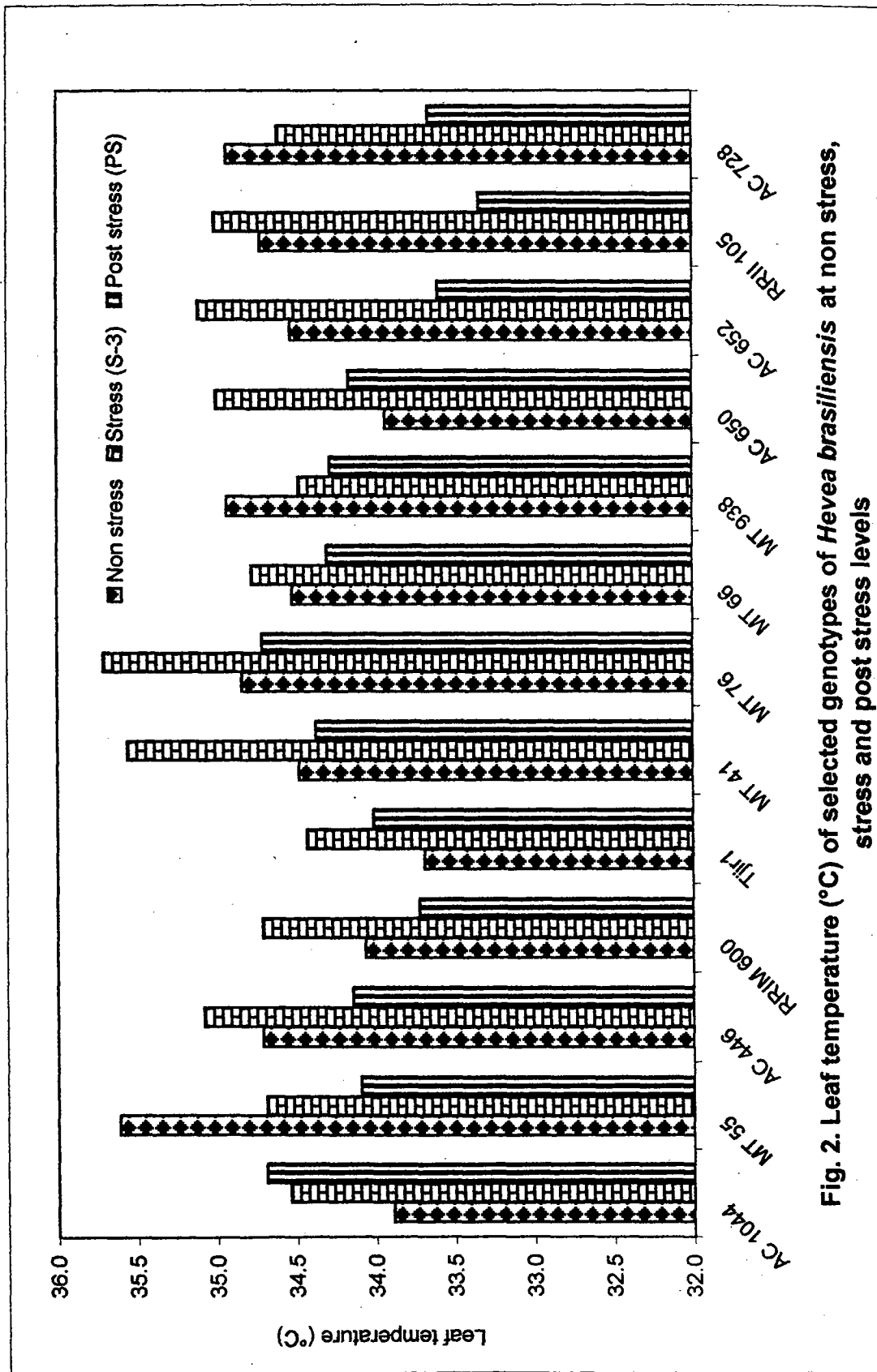


Fig. 2. Leaf temperature (°C) of selected genotypes of *Hevea brasiliensis* at non stress, stress and post stress levels

Table 5. Result of Factorial CRD for leaf temperature (°C) of *Hevea* genotypes during non stress (NS) and stress (S-3) periods

Genotype	Non stress (NS)	Stress (S-3)	Mean	Conclusion	
AC 1044	33.89	34.54	34.22	SE plot ⁻¹	0.094
MT 55	35.61	34.69	35.15	Gen. Mean	34.714
AC 446	34.71	35.08	34.89	CV %	1.69
RRIM 600	34.06	34.71	34.38	Variance ratio	
Tjir1	33.69	34.43	34.06	Genotype	2.197*
MT 41	34.48	35.56	35.01	NS vs S	7.868**
MT 76	34.84	35.71	35.28	G x NS vs S	1.602
MT 66	34.52	34.78	34.65	CD (P=0.05)	
MT 938	34.93	34.48	34.71	Genotype	0.569
AC 650	33.93	35.00	34.47	NS vs S	0.223
AC 652	34.53	35.11	34.82	Interact	0.804
RRII 105	34.72	35.01	34.87		
AC 728	34.93	34.61	34.77		
Mean	34.53	34.9			

Analysis of data was done separately for each level of water stress with non-stress, in order to assess genotypic variability, effect of water stress, as well as the interaction effect between genotype x non-stress vs stress period (Table 14). Leaf temperature was found to be significantly more during stress period. When S-2 and post-stress periods were considered with non-stress period, significant difference was noticed only between non-stress vs stress period and not between genotypes or the interaction between genotype x non-stress vs stress period. On the other hand when stress (S-3) was considered (Table 5) with non-stress, there was significant difference in the leaf temperature between the genotypes as well as non-stress vs stress period.

Corresponding to stress periods, the non-stress period of plants under irrigation was considered in order to nullify the effect of age difference between the plants (Table 14). The non-stress (NS-2) was taken against S-2, NS-3 against S-3 and NS-4 against post stress. When NS-2 and S-2 were considered as non-stress and stress periods, there was significant difference in the leaf temperature between non-stress vs stress period, but not among genotypes or the interaction between genotype x non-stress vs stress period. But the effect was different when NS-3 and S-3 were considered as non-stress and stress periods. Here, the differences in leaf temperature between the genotypes, non-stress vs stress period as well as the interaction between genotypes x non-stress vs stress period were significant. The response was different when NS-4 was compared with post-stress leaf temperature of stressed plants. There was significant genotypic difference as well as significant interaction between the genotypes x non-stress vs post stress period, but no significant difference between non-stress vs post stress period.

4.2.2 Components of water relation

Stomatal conductance, transpiration rate and leaf water potential are considered here.

4.2.2.1. Stomatal conductance

The stomatal conductance under various water levels is given in Table 6. Under non-stress condition, the mean stomatal conductance was $0.941 \text{ moles m}^{-2}\text{s}^{-1}$ with a range of $0.353 - 1.749 \text{ moles m}^{-2}\text{s}^{-1}$. After a period of one month water stress (stress-2) when the stomatal conductance was recorded, the mean value was $1.061 \text{ moles m}^{-2}\text{s}^{-1}$ with a minimum stomatal conductance of 0.575 and a maximum of $1.683 \text{ moles m}^{-2}\text{s}^{-1}$. When the water stress intensity was further increased (S-3), the mean stomatal conductance was reduced to $0.807 \text{ moles m}^{-2}\text{s}^{-1}$ and the minimum conductance was 0.482 with a maximum conductance of $1.725 \text{ moles m}^{-2}\text{s}^{-1}$. After giving 2 days irrigation when the post stress effect was studied, the mean stomatal conductance showed an increase to 1.998 and the range was $1.486 - 2.876 \text{ moles m}^{-2}\text{s}^{-1}$.

Analysis of data done separately for each water level, clearly indicated significant genotypic differences. Under non-stress condition, the genotype AC 652 recorded the lowest stomatal conductance, whereas the highest was in the clone Tjir1. When the stress intensity increased, the response of the genotypes was different, where the minimum conductance was recorded by the genotype AC 1044 and the maximum by MT 66. When the stress intensity was further increased, stomatal conductance was reduced to the minimum in the genotype MT 41 and MT 938 recorded the maximum. Under post stress condition, the minimum stomatal conductance was recorded by MT 66 and the clone RRIM 600 recorded the maximum conductance. The response of each genotype under non-stress, stress and post stress levels is represented in Fig. 3. The genotypes MT 66, MT 938, AC 650 and AC 652 were found to be not responsive to water stress levels indicated by the increased rate of stomatal conductance at water stress level.

Table 6. Stomatal conductance (moles $m^{-2} s^{-1}$) of selected genotypes of *Hevea brasiliensis* at varying levels of water stress

Genotype	Non stress (NS)	Stress (S-2)	Stress (S-3)	Post stress (PS)
AC 1044	0.648 ^{cd}	0.575 ^e	0.507 ^g	1.608 ^{de}
MT 55	0.618 ^{cd}	0.604 ^e	0.541 ^{fg}	1.580 ^{de}
AC 446	0.809 ^c	0.715 ^{de}	0.543 ^{fg}	1.227 ^e
RRIM 600	1.453 ^b	1.130 ^{bc}	0.732 ^{de}	2.876 ^a
Tjir1	1.749 ^a	1.651 ^a	0.809 ^d	1.827 ^{cde}
MT 41	0.650 ^{cd}	0.617 ^e	0.482 ^g	2.048 ^{bode}
MT 76	1.176 ^b	1.079 ^{bc}	0.982 ^c	2.250 ^{abcd}
MT 66	0.670 ^{cd}	1.683 ^a	1.091 ^{bc}	1.486 ^{de}
MT 938	0.830 ^c	1.267 ^b	1.725 ^a	1.932 ^{cde}
AC 650	0.696 ^c	0.949 ^{cd}	0.698 ^{def}	2.164 ^{abcd}
AC 652	0.353 ^d	0.935 ^{cd}	0.631 ^{efg}	2.845 ^{ab}
RRII 105	1.304 ^b	0.920 ^{cd}	0.499 ^g	1.588 ^{de}
AC 728	1.272 ^b	1.673 ^a	1.248 ^b	2.545 ^{abc}
Mean	0.941	1.061	0.807	1.998

Any two means having a common letter are not significantly different

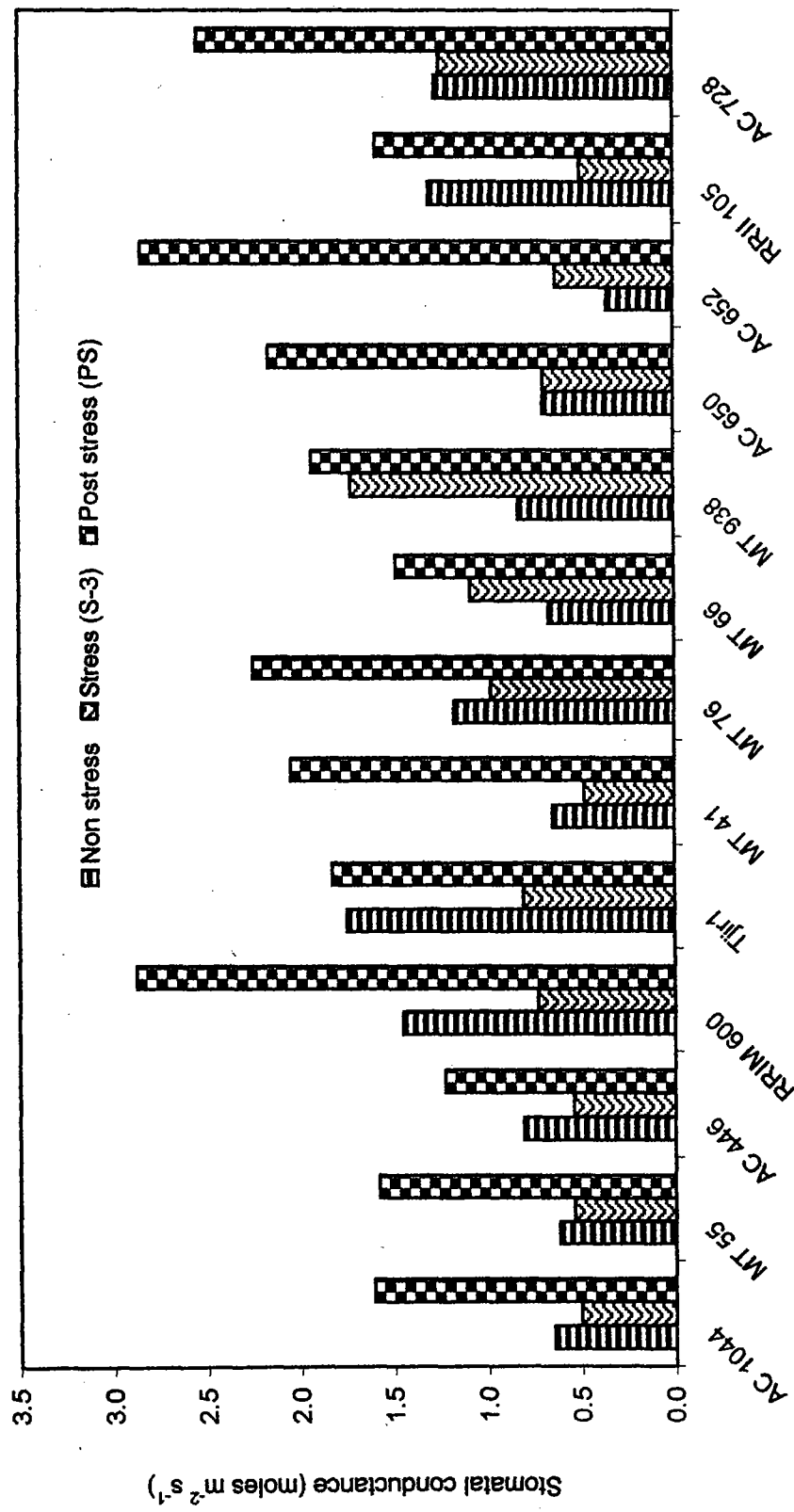


Fig. 3. Stomatal conductance (moles m⁻² s⁻¹) of selected genotypes of *Hevea brasiliensis* at non stress, stress and post stress levels

Table 7. Result of Factorial CRD for stomatal conductance (moles $m^{-2}s^{-1}$) of *Hevea* genotypes during non stress (NS) and stress (S-3) periods

Genotype	Non stress (NS)	Stress (S-3)	Mean	Conclusion	
AC 1044	0.648	0.507	0.578	SE plot ¹	0.023
MT 55	0.618	0.541	0.580	Gen. Mean	0.874
AC 446	0.809	0.543	0.676	CV %	16.11
RRIM 600	1.453	0.732	1.093	Variance ratio	
Tjir1	1.749	0.809	1.279	Genotype	26.56**
MT 41	0.650	0.482	0.566	NS vs S	17.63**
MT 76	1.176	0.982	1.079	G x NS vs S	19.17**
MT 66	0.670	1.091	0.880	CD (P=0.05)	
MT 938	0.830	1.725	1.278	Genotype	0.136
AC 650	0.696	0.698	0.697	NS vs S	0.053
AC 652	0.353	0.631	0.492	Interact	0.193
RRII 105	1.304	0.499	0.902		
AC 728	1.272	1.248	1.260		
Mean	0.941	0.807			

To know the effect of each level of water stress on the genotypes, analysis of data was done separately by taking non-stress level with each of the stress levels and with the post stress level (Table 14). When non-stress with S-2 stress level and post-stress were considered separately, genotypic difference, difference between non-stress and stress and the interaction effect between genotype x non-stress vs stress were all significant. Similar was the result when non-stress period was considered against S-3 stress level (Table 7).

The various stress levels of plants under water stress were analysed statistically with the corresponding non-stress periods of plants under irrigation (Table 14). When S-2 was compared with NS-2, there was significant genotypic difference as well as significant interaction effect between genotype x NS vs S, but the difference between NS vs S was not significant. Under increased stress intensity (NS-3 vs S-3) the difference was significant for all the conditions. The genotype, the stress levels (non-stress and stress) and the interaction between genotype and stress levels differed significantly. When NS-4 was compared with post stress period, genotypic difference and interaction effect were significant.

4.2.2.2 Transpiration rate (inside the chamber)

Transpiration rate of selected genotypes at varying levels of water stress is shown in Table 8. Under non-stress condition, the mean transpiration rate recorded was $20.55 \mu\text{g cm}^{-2} \text{s}^{-1}$ with a range of $8.62 - 35.47 \mu\text{g cm}^{-2} \text{s}^{-1}$. When the water stress intensity increased to S-2 level, the mean transpiration rate was reduced to $18.35 \mu\text{g cm}^{-2} \text{s}^{-1}$ with a minimum transpiration of $10.0 \mu\text{g cm}^{-2} \text{s}^{-1}$ and a maximum of $26.19 \mu\text{g cm}^{-2} \text{s}^{-1}$. When the stress intensity was increased further (at S-3) the genotypic response to water stress was different. At this level, the minimum transpiration rate recorded was $10.87 \mu\text{g cm}^{-2} \text{s}^{-1}$ whereas the maximum was $40.49 \mu\text{g cm}^{-2} \text{s}^{-1}$ and the mean value recorded was $18.34 \mu\text{g cm}^{-2} \text{s}^{-1}$. At

Table 8. Transpiration rate ($\mu\text{g cm}^{-2} \text{s}^{-1}$) of selected genotypes of *Hevea brasiliensis* at varying levels of water stress

Genotype	Non stress (NS)	Stress (S-2)	Stress (S-3)	Post stress (PS)
AC 1044	13.85 ^d	12.38 ^{fg}	10.87 ^f	28.41 ^d
MT 55	15.56 ^{cd}	14.94 ^{ef}	12.92 ^{def}	34.87 ^{abcd}
AC 446	20.29 ^c	16.49 ^{def}	13.75 ^{def}	27.16 ^d
RRIM 600	25.17 ^b	19.87 ^{bcd}	16.87 ^{cde}	40.45 ^{ab}
Tjir1	35.47 ^a	21.39 ^{bc}	18.23 ^{cd}	29.19 ^{cd}
MT 41	15.03 ^d	10.00 ^g	15.45 ^{cdef}	38.39 ^{abcd}
MT 76	26.97 ^b	19.91 ^{bcd}	19.50 ^c	34.01 ^{bcd}
MT 66	15.23 ^d	24.14 ^{ab}	20.69 ^c	30.90 ^{bcd}
MT 938	18.12 ^{cd}	20.64 ^{bcd}	30.50 ^b	36.14 ^{abcd}
AC 650	16.56 ^{cd}	20.99 ^{bcd}	13.70 ^{def}	32.19 ^{bcd}
AC 652	8.62 ^e	14.72 ^{ef}	12.77 ^{ef}	45.45 ^a
RRII 105	28.83 ^b	16.90 ^{cde}	12.66 ^{ef}	27.42 ^d
AC 728	27.41 ^b	26.19 ^a	40.49 ^a	39.74 ^{abc}
Mean	20.55	18.35	18.34	34.18

Any two means having a common letter are not significantly different

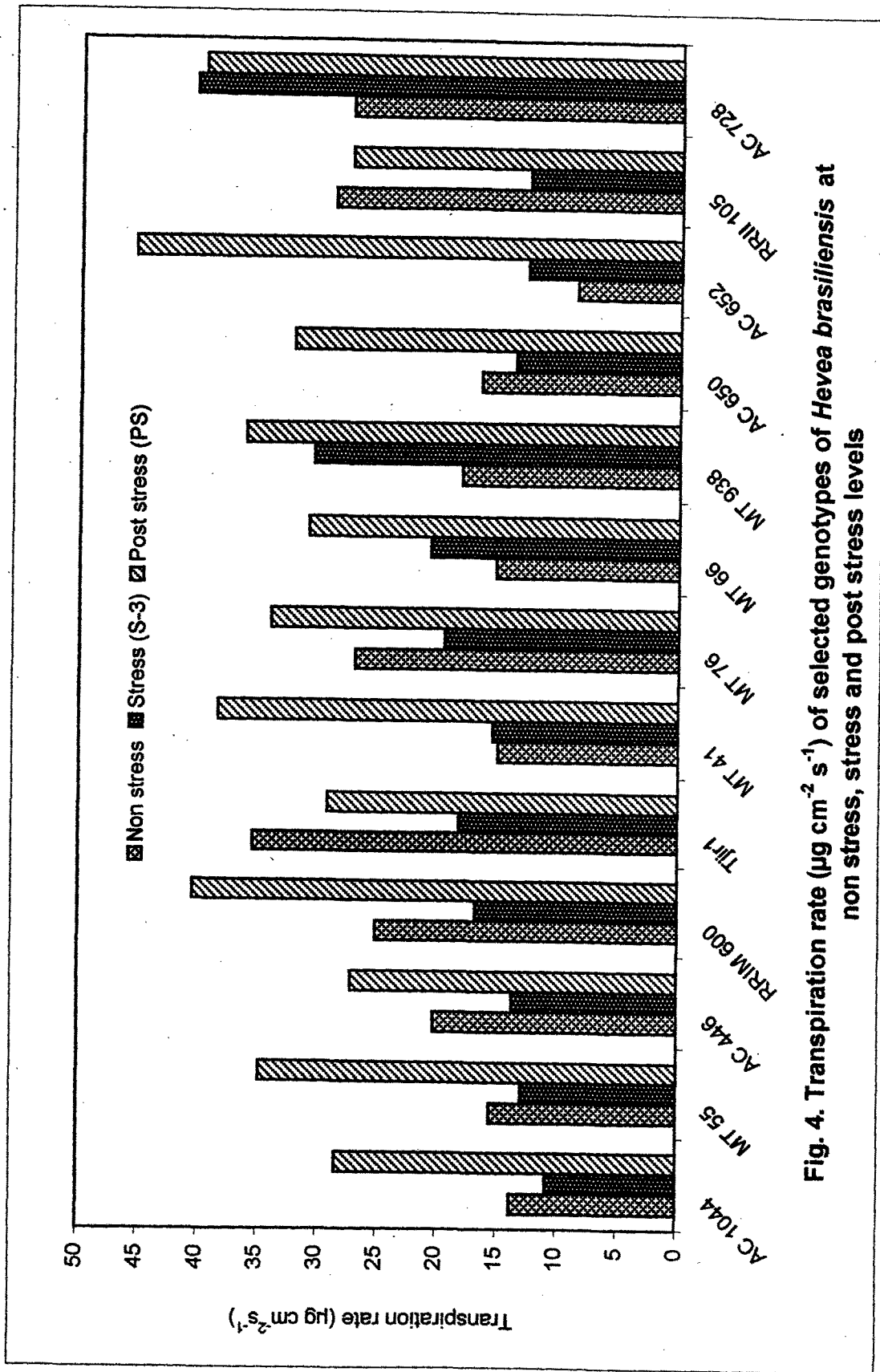


Fig. 4. Transpiration rate ($\mu\text{g cm}^{-2} \text{s}^{-1}$) of selected genotypes of *Hevea brasiliensis* at non stress, stress and post stress levels

Table 9. Result of Factorial CRD for transpiration rate ($\mu\text{g cm}^{-2} \text{s}^{-1}$) of *Hevea* genotypes during non stress (NS) and stress (S-3) periods

Genotype	Non stress (NS)	Stress (S-3)	Mean	Conclusion	
AC 1044	13.85	10.87	2.36	SE plot ⁻¹	0.441
MT 55	15.56	12.92	14.24	Gen. Mean	19.44
AC 446	20.29	13.75	17.02	CV %	14.16
RRIM 600	25.17	16.87	21.02	Variance ratio	
Tjir1	35.47	18.23	26.85	Genotype	33.38**
MT 41	15.03	15.45	15.24	NS vs S	12.54**
MT 76	26.97	19.50	23.24	G x NS vs S	17.46**
MT 66	15.23	20.69	17.96	CD (P=0.05)	
MT 938	18.12	30.50	24.31	Genotype	2.667
AC 650	16.56	13.70	15.13	NS vs S	1.044
AC 652	8.62	12.77	10.69	Interact	3.77
RRII 105	28.83	12.66	20.75		
AC 728	27.41	40.49	33.96		
Mean	20.55	18.34			

post stress level, the response of genotypes was again different. Here, the minimum recorded was $27.16 \mu\text{g cm}^{-2} \text{s}^{-1}$ with a maximum transpiration rate of $45.45 \mu\text{g cm}^{-2} \text{s}^{-1}$ and a mean value of $34.18 \mu\text{g cm}^{-2} \text{s}^{-1}$.

Genotypic performance under each water level was statistically analysed, and the result indicated significant genotypic difference under each level. Under non-stress condition, the lowest transpiration rate was recorded by the wild genotype AC 652, which was lower than that of all the standard clones considered for the study. However, the highest rate of transpiration was occurred in the drought susceptible clone Tjir1, which further confirms its drought susceptibility. When stress level was increased to S-2 after a period of one month water stress, the genotypic response was different as in the case of stomatal conductance. At this level, the minimum transpiration rate was recorded in the wild genotype MT 41 ($15.03 \mu\text{g cm}^{-2} \text{s}^{-1}$), which was significantly lower than that of the drought tolerant clone RRIM 600 and that of standard clone RRII 105, where the rate of transpiration was 19.87 and $16.9 \mu\text{g cm}^{-2} \text{s}^{-1}$ respectively. But when the stress intensity was increased further, the genotypic response again changed. At this level (S-3) the maximum control on transpirational loss of water was expressed by the wild genotype AC 1044 with a minimum transpiration rate of $10.87 \mu\text{g cm}^{-2} \text{s}^{-1}$ whereas the water control was minimum in the wild genotype AC 728, which was having a maximum transpiration rate of $40.49 \mu\text{g cm}^{-2} \text{s}^{-1}$. But the standard clones reacted differently, where the transpiration rate of RRII 105 was significantly lesser than the drought tolerant clone RRIM 600 and the drought susceptible clone Tjir1. Under post stress level, all the genotypes exhibited a higher rate of transpiration. However, the maximum was recorded by the wild genotype AC 652, followed by the clone RRIM 600 and the minimum transpiration rate was observed in the wild genotype AC446 followed by the clone RRII 105.

Figure 4 gives a clear picture of genotypic response for rate of transpiration under non-stress, stress and post stress levels. Except for genotypes MT 41, MT66, MT 938, AC 652 and AC 728 all other genotypes exhibited a drastic reduction of transpiration rate under severe water stress condition. This indicates their capability of keeping a well controlled water balance system, by an efficient stomatal closure mechanism.

As in the previous cases, here also genotypic performance was statistically analysed by considering the non-stress level with each water stress level of the stressed plants (Table 14). When non-stress and S-2 stress level of stressed plants were considered, the genotypic difference, NS vs S and genotype x NS vs S were all significantly different. Similar was the result when the water levels NS and post stress were considered. The genotypic response and the effect of NS vs S-3 stress levels are given in Table 9.

Followed by this, the response between stressed plants and irrigated plants were analysed by considering the various stress levels and corresponding non-stress levels, i.e., NS-2 vs S-2, NS-3 vs S-3, and NS-4 vs PS (Table 14). Here, under all levels between non-stress and stress/post stress condition, the genotypic difference, non-stress vs stress/post stress and the interaction of genotype x NS vs S were significantly different.

4.2.2.3 Leaf water potential

The mean leaf water potential (Ψ leaf) of selected genotypes under various water levels are shown in Table 9. Under non-stress condition the mean Ψ leaf recorded was -2.92 MPa with a range of -3.15 to -2.74 MPa. After 15 days of stress period, the Ψ leaf was slightly affected with a mean value of -2.92 MPa, but the range varied from -3.23 to -2.7 MPa. At S-2 level also there was much difference in the mean Ψ leaf recorded. The Ψ leaf varied from -3.44 to

Table 10. After noon leaf water potential (-MPa) of selected genotypes of *Hevea brasiliensis* at varying levels of water stress

Genotype	Non stress (NS)	Stress (S-1)	Stress (S-2)	Stress (S-3)	Post stress (PS)
AC 1044	-3.000 ^a	-3.076 ^{bcd}	-3.228 ^{de}	-3.504 ^a	-2.986 ^b
MT 55	-2.891 ^a	-2.781 ^{ab}	-2.815 ^{bc}	-2.951 ^a	-2.970 ^b
AC 446	-2.776 ^a	-2.800 ^{ab}	-2.908 ^{bcd}	-3.132 ^a	-2.919 ^b
RRIM 600	-2.801 ^a	-2.841 ^{ab}	-3.199 ^{de}	-3.464 ^a	-3.071 ^b
Tjir1	-2.777 ^a	-2.702 ^a	-2.603 ^{ab}	-3.076 ^a	-2.711 ^{ab}
MT 41	-2.759 ^a	-2.869 ^{abc}	-2.426 ^a	-2.527 ^a	-2.197 ^a
MT 76	-3.220 ^a	-3.238 ^d	-3.443 ^e	-2.808 ^a	-3.141 ^b
MT 66	-3.349 ^a	-3.199 ^d	-3.169 ^{de}	-3.428 ^a	-3.179 ^b
MT 938	-2.920 ^a	-2.847 ^{ab}	-2.659 ^{ab}	-2.678 ^a	-2.187 ^a
AC 650	-2.769 ^a	-3.200 ^{cd}	-3.257 ^{de}	-3.533 ^a	-3.169 ^b
AC 652	-2.792 ^a	-2.864 ^{abc}	-2.313 ^a	-2.699 ^a	-2.152 ^a
RRII 105	-2.737 ^a	-2.785 ^{ab}	-3.107 ^{ode}	-3.337 ^a	-2.898 ^b
AC 728	-3.154 ^a	-2.781 ^{ab}	-2.625 ^{ab}	-3.015 ^a	-2.503 ^{ab}
Mean	-2.919	-2.922	-2.904	-3.089	-2.776

Any two means having a common letter are not significantly different

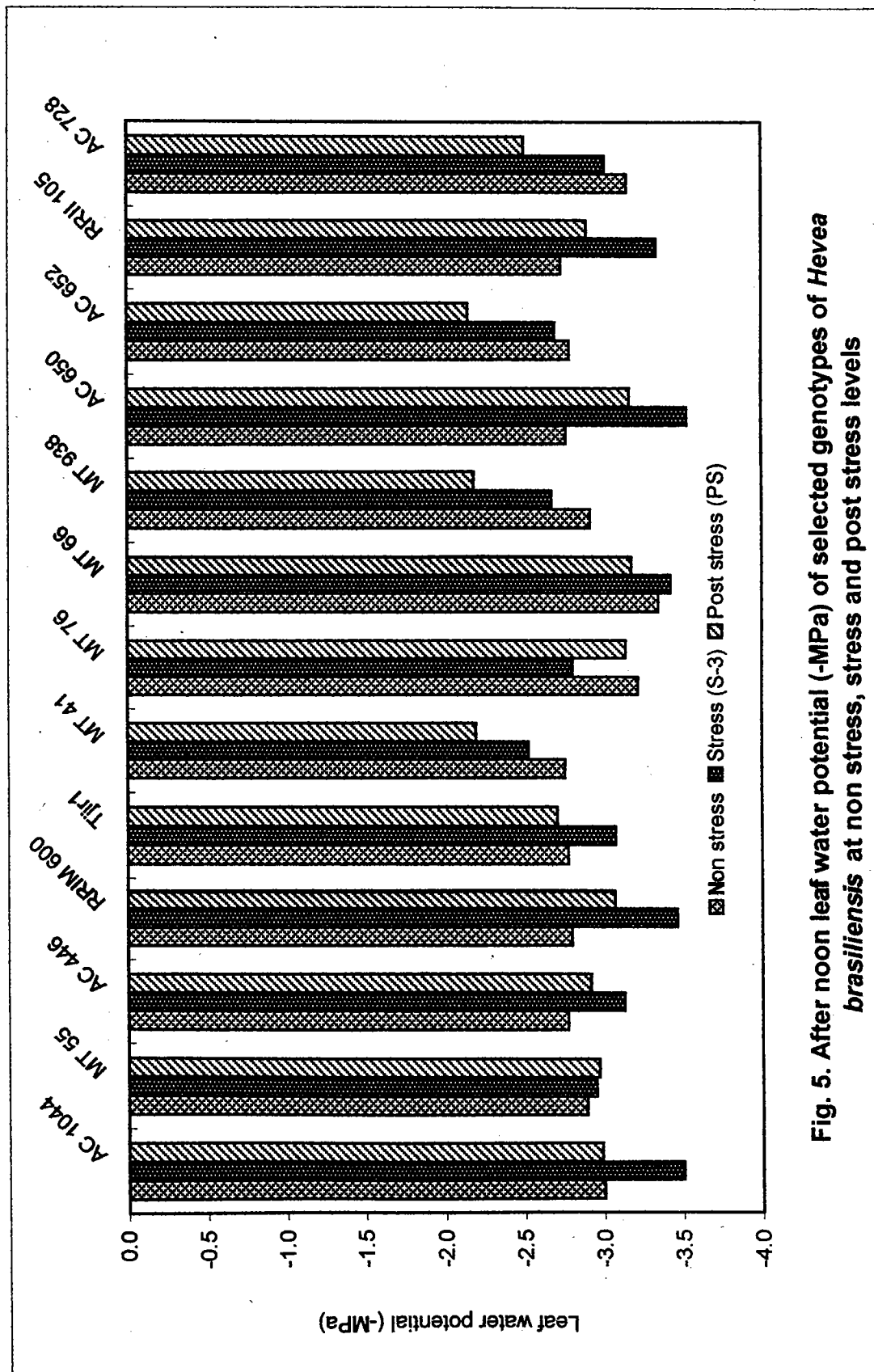


Fig. 5. After noon leaf water potential (-MPa) of selected genotypes of *Hevea brasiliensis* at non stress, stress and post stress levels

Table 11. Result of Factorial CRD for after noon leaf water potential (-MPa) of *Hevea* genotypes during non stress (NS) and stress (S-3) periods

Genotype	Non stress (NS)	Stress (S-3)	Mean	Conclusion	
AC 1044	-3.000	-3.504	-3.252	SE plot ⁻¹	0.0692
MT 55	-2.891	-2.951	-2.921	Gen. Mean	-3.004
AC 446	-2.776	-3.132	-2.954	CV %	-14.390
RRIM 600	-2.801	-3.464	-3.133	Variance ratio	
Tjir1	-2.777	-3.076	-2.926	Genotype	1.3634
MT 41	-2.759	-2.527	-2.643	NS vs S	3.0081
MT 76	-3.220	-2.808	-3.014	G x NS vs S	1.2035
MT 66	-3.349	-3.428	-3.389	CD (P=0.05)	
MT 938	-2.920	-2.678	-2.799	Genotype	0.4188
AC 650	-2.769	-3.533	-3.151	NS vs S	0.1642
AC 652	-2.792	-2.699	-2.746	Interact	0.5923
RRII 105	-2.737	-3.337	-3.037		
AC 728	-3.154	-3.015	-3.085		
Mean	-2.919	-3.089			

-2.31 MPa with the mean value of -2.9 MPa. When the stress intensity was increased to S-3 level, the mean Ψ leaf was reduced to -3.09 MPa with a range of -3.53 to -2.53 MPa. When irrigation was given after a stress period of 45 days, the genotypes responded well, where the mean Ψ leaf was increased to -2.78 MPa with a minimum of -3.18 MPa and a maximum of -2.15 MPa.

Data were analysed statistically for knowing the Ψ leaf at each level. There was no significant genotypic difference for Ψ leaf under non-stress condition. However, the clone RRII 105 showed the highest Ψ leaf followed by the genotype MT 41. The lowest Ψ leaf was recorded in the genotype MT 66, followed by MT 76. But under S-1 period, the difference among the genotypes was significant with the highest Ψ leaf shown by the clone Tjir1 and the lowest by MT 76. The Ψ leaf of the clones RRIM 600 and RRII 105 was on par with each other and the genotype AC 652 was having more Ψ leaf than this two clones. Under S-2 level also, the genotypic difference for Ψ leaf was significant, where the genotype AC 652 recorded the highest Ψ leaf followed by MT 41 and the lowest by the genotype MT 76. At this stress level, the Ψ leaf of the clones RRIM 600 and RRII 105 was lower than Tjir1. This was repeated under S-3 period also, but there was no significant genotypic difference. Under this stress period, the genotype MT 41 showed highest Ψ leaf and the lowest was by AC 650. Under post-stress level, the difference in Ψ leaf among the genotypes was significant and the genotype AC 652 recorded the highest Ψ leaf, whereas the lowest was by the genotype MT 66. The clones RRIM 600, Tjir1 and RRII 105 responded similarly. This response of Ψ leaf under various water levels is clear from the Fig. 5.

The effect of each water stress level with the non-stress level was analysed separately (Table 14) in order to evaluate the genotypic response, water

level effect as well as the interaction effect. When the Ψ leaf at NS was compared with S-1 level, only the genotypic difference was significant, whereas NS vs S and the interaction effect were not significant. When NS and S-2 levels were considered, the genotypic as well as interaction between genotype x NS vs S were significant, but NS vs S was not significant. On the other hand when NS and S-3 levels were considered, there was no significant difference for any of the factor or for the interaction and for NS and PS levels the significant difference was only for genotypes.

The stress periods were then compared with corresponding non-stress periods of irrigated plants (Table 14). There was significant genotypic difference, difference between NS vs S and the interaction effect between genotype x NS vs S, when NS-1 and S-1 as well as NS-2 and S-2 levels were compared. When NS-3 and S-3 levels were compared, the effect was significant for NS vs S and for interaction, but NS-4 and PS levels exhibited significant difference between genotypes, between NS vs S periods and genotype x NS vs S interaction effect.

4.2.2.4. Soil water potential

Soil water potential (Ψ soil) recorded for each genotype under varying water levels and the mean Ψ soil are shown in Table 12. Under non-stress condition, the mean Ψ soil recorded was -0.802 MPa with a range of -1.77 to -0.314 MPa. When the water stress increased to S-1 level, the mean Ψ soil reduced to -1.36 MPa and the range observed was -2.98 to -0.80 MPa and with increasing stress intensity (at S-2) the mean Ψ soil further reduced to -3.1 MPa and the range varied from -5.16 to -1.83 MPa. At water stress S-3, there was a further reduction of Ψ soil to -4.13 MPa and the minimum Ψ soil recorded was -6.79 MPa whereas the maximum Ψ soil was -2.1 MPa. At post stress level there was an increase of Ψ soil for all the genotypes, where the mean Ψ soil increased to -3.08 MPa with a range of -5.12 to -1.14 MPa.

Table 12. After noon soil water potential (-MPa) of selected genotypes of *Hevea brasiliensis* at varying levels of water stress

Genotype	Non stress (NS)	Stress (S-1)	Stress (S-2)	Stress (S-3)	Post stress (PS)
AC 1044	-0.979 ^f	-1.172 ^b	-3.053 ^{bcd}	-3.760 ^{abc}	-2.491 ^{ab}
MT 55	-0.490 ^{bc}	-0.904 ^a	-2.852 ^{bc}	-3.430 ^{ab}	-2.98 ^{abc}
AC 446	-0.660 ^e	-0.908 ^a	-3.151 ^{bcd}	-3.653 ^{ab}	-2.472 ^{ab}
RRIM 600	-0.697 ^e	-0.853 ^a	-1.827 ^a	-2.908 ^a	-2.343 ^{ab}
Tjir1	-0.552 ^{cd}	-1.084 ^b	-3.057 ^{bcd}	-3.417 ^{ab}	-1.141 ^a
MT 41	-1.327 ^g	-1.900 ^d	-3.096 ^{bcd}	-3.753 ^{abc}	-2.906 ^{abc}
MT 76	-0.480 ^b	-1.494 ^c	-2.963 ^{bc}	-4.704 ^{cd}	-3.019 ^{abc}
MT 66	-0.469 ^{bc}	-1.616 ^c	-2.899 ^{bc}	-4.919 ^d	-4.285 ^{bc}
MT 938	-0.612 ^{de}	-1.545 ^c	-5.161 ^e	-6.786 ^e	-5.115 ^c
AC 650	-1.067 ^f	-1.232 ^b	-3.648 ^d	-4.145 ^{bcd}	-2.886 ^{abc}
AC 652	-1.766 ^h	-2.982 ^e	-3.225 ^{cd}	-4.285 ^{bcd}	-3.649 ^{bc}
RRII 105	-1.049 ^f	-1.158 ^b	-2.925 ^{bc}	-3.984 ^{bcd}	-3.399 ^{abc}
AC 728	-0.314 ^a	-0.796 ^a	-2.517 ^b	-3.936 ^{bcd}	-3.296 ^{abc}
Mean	-0.802	-1.357	-3.106	-4.129	-3.076

Any two means having a common letter are not significantly different

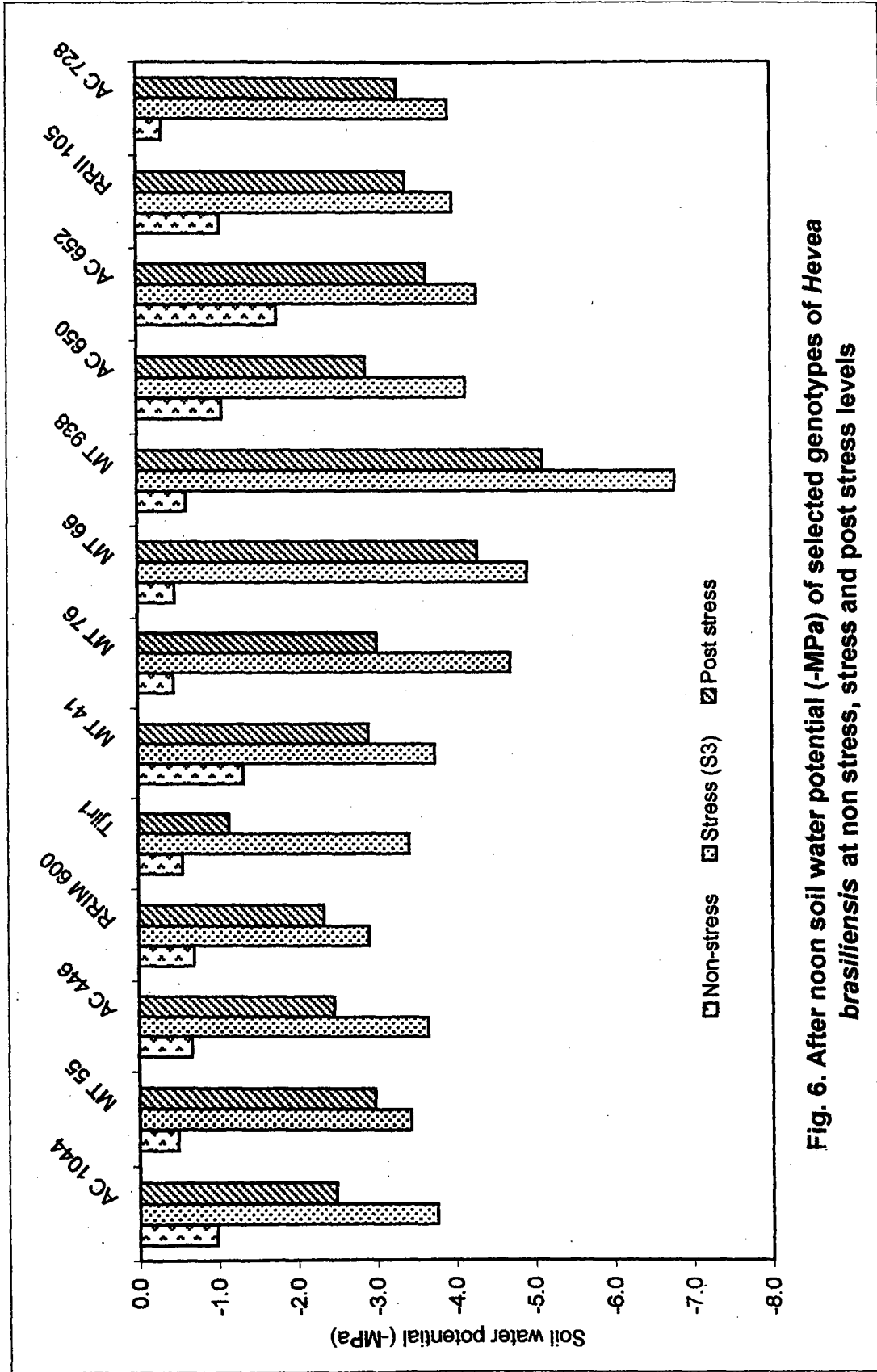


Fig. 6. After noon soil water potential (-MPa) of selected genotypes of *Hevea brasiliensis* at non stress, stress and post stress levels

Table 13. Result of Factorial CRD for after noon soil water potential (-MPa) of *Hevea* genotypes during non stress (NS) and stress (S-3) periods

Genotype	Non stress (NS)	Stress (S-3)	Mean	Conclusion	
AC 1044	-0.979	-3.760	-2.369	SE plot ⁻¹	0.598
MT 55	-0.490	-3.430	-1.960	Gen. Mean	-2.466
AC 446	-0.660	-3.653	-2.157	CV %	15.14
RRIM 600	-0.697	-2.908	-1.802	Variance ratio	
Tjir1	-0.552	-3.417	-1.985	Genotype	11.02**
MT 41	-1.327	-3.753	-2.539	NS vs S	1547.87**
MT 76	-0.448	-4.704	-2.576	G x NS vs S	12.56**
MT 66	-0.469	-4.919	-2.694	CD (P=0.05)	
MT 938	-0.612	-6.786	-3.699	Genotype	3.616
AC 650	-1.067	-4.145	-2.606	NS vs S	1.419
AC 652	-1.766	-4.285	-3.026	Interact	5.116
RRII 105	-1.049	-3.984	-2.517		
AC 728	-0.314	-3.936	-2.125		
Mean	-0.802	-4.129			

Table 14. Summary anova (F value) for various physiological parameters as a response of *Hevea* genotypes to moisture stress

Parameter	NS vs S-1	NS vs S-2	NS vs S-3	NS vs PS	NS-1 vs S-1	NS-2 vs S-2	NS-3 vs S-3	NS-4 vs PS
Leaf temperature								
G		0.9398	2.1971*	1.4491		1.7576	6.2470**	9.8249**
NS vs S	-	17.1117**	7.8675**	9.1053**	-	8.0603**	6.6579*	0.5307
G x NS vs S		1.4174	1.6021	1.7310		1.7175	5.8631**	13.6276**
Stomatal conductance								
G		27.1062**	26.5662**	6.8767**		26.2965**	10.8134*	6.5983**
NS vs S	-	10.1747**	17.6322**	201.4520**	-	1.1819	229.3351**	1.8931
G x NS vs S		8.4237**	19.1678**	5.2796**		13.3546**	17.4546**	6.4737**
Transpiration rate								
G		26.0685**	33.3764**	4.6629**		12.1255*	11.4490**	6.6517**
NS vs S	-	14.4058**	12.5404**	179.776**	-	11.6876**	18.9408**	10.7175**
G x NS vs S		10.3902**	17.4599**	8.7420**		6.9105**	10.9977**	3.8038**
Ψ leaf								
G	2.0213*	3.6999**	1.3634	2.5958**	13.3247**	21.4082**	1.9257	4.6750**
NS vs S	0.0021	0.0476	3.0081	2.9148	238.5327**	575.8277**	266.3211**	525.86**
G x NS vs S	0.5696	1.9738*	1.2035	1.5942	6.3918**	18.7360**	3.5088**	7.1961**
Ψ soil								
G	213.0435**	21.4952**	11.0233**	36.5910**	158.5299**	17.7094**	10.2682**	25.6297**
NS vs S	966.4468**	1850.44**	1547.87**	4110.98**	928.06**	1864.41**	1655.57**	4041.87**
G x NS vs S	38.8858**	17.6066**	12.5615**	44.42**	35.4274**	14.9243**	10.6021**	30.5875**

* - Significant at $P = 0.05$

** - Significant at $P = 0.01$

Analysis of data done separately for each water level indicated significant genotypic differences for Ψ soil and at post stress level, the difference was insignificant. Under non-stress condition, as well as at S-1 stress level, the maximum Ψ soil was shown by the genotype AC 728, whereas the genotype AC 652 showed the minimum Ψ soil. But at S-2 and S-3 stress levels, clone RRIM 600 was having the maximum Ψ soil and the genotype MT 938 was showing the minimum Ψ soil. At post stress level the quick response was by the clone Tjir1 and the genotype MT 938 showed least response to irrigation. Fig. 6 exhibits the genotypic difference in Ψ soil at different water levels.

Significance of genotypic difference, stress effect and the interaction effect of genotype x NS vs S were tested statistically for each water level separately (Table 14). When NS level was taken with S-1 and S-2 levels separately, there was significant difference between the genotypes, between NS vs S periods and genotype x NS vs S interaction effect. The same was the result when NS vs S-3 (Table 13) were considered. When NS level was taken against PS level the genotypic difference as well as the interaction effect was significant at 5 per cent level whereas the difference between NS vs PS was significant even at 1 per cent level.

Followed by this analysis, the effect of water stress was analysed by considering the water levels under controlled condition, i.e., NS-1 with S-1, NS-2 with S-2, NS-3 with S-3 and NS-4 with PS of the stressed plants (Table 14). All the stress levels showed significant difference among genotypes, between NS vs S, and the interaction effect between genotype x NS vs S when considered with corresponding non-stress condition of the irrigated plants. While NS-4 was taken against PS level of stressed plants, there was significant difference only for the water levels considered and there was no genotypic difference or interaction effect.

4.2.3 Chlorophyll fluorescence

The various components of chlorophyll fluorescence signals emitted (i.e., initial fluorescence (F_0), maximal fluorescence (F_m) and variable fluorescence (F_v) along with ratio F_v/F_m and F_m/F_0 of the selected genotype at non-stress as well as stress conditions are shown in Table 15. The F_0 of genotypes under non-stress condition ranged from 164.78 – 220.78 with a mean value of 194.67, whereas at stress condition the F_0 increased in all the genotypes and the range was between 417.56 – 629.0 with a mean of 556.98. The maximal fluorescence (F_m) under non-stress condition was in the range of 623.45 – 820.11 and the mean F_m was 719.43. At stress level this F_m was reduced to 357.11 and ranged from 235.0 – 500.89. The variable fluorescence (F_v) under non-stress condition ranged from 438.56 – 628.67 and the mean F_v recorded was 524.84. As in the case of F_m , F_v also reduced to 162.44 under stress condition and the value ranged from 70.22 – 288.22. The F_v/F_m ratio under non-stress condition was 0.728 and ranged from 0.693 – 0.769 whereas at stress level the mean ratio was reduced to 0.438 and the range was 0.299 – 0.575. Similarly the ratio F_m/F_0 under non-stress level was higher than that under stress condition. The mean F_m/F_0 at non-stress level was 3.717 and the range was 3.258 - 4.328, whereas under stress condition, the F_m/F_0 dropped to 0.653 and the range occurred was between 0.405 – 0.942.

The data were analysed statistically and for all the components of fluorescence there was significant genotypic difference both under stress and non-stress levels and this was true for the ratios F_v/F_m and F_m/F_0 . The drastic increase of F_0 under stress level is clearly illustrated in Fig. 7. Figs. 8 to 11 represent the drastic reduction noticed in the genotypes for F_m , F_v , F_v/F_m and F_m/F_0 respectively.

The data were statistically analysed for non-stress and stress levels as well as for the interaction between genotype and NS vs S levels. Table 16 gives the result of the above analysis for F_0 where there was significant difference

Table 15. Fluorescence parameters recorded in the selected genotypes of *Hevea brasiliensis* under non stress (NS) and stress (S) condition

Genotype		F_o	F_m	F_v	F_v/F_m	F_m/F_o
AC 1044	NS	212.67	820.00	607.33	0.741	3.857
	S	531.78	500.89	288.22	0.575	0.942
MT 55	NS	189.33	818.00	628.67	0.769	4.328
	S	583.78	423.55	234.22	0.553	0.726
AC 446	NS	220.78	736.56	515.78	0.700	3.337
	S	526.89	430.45	209.67	0.487	0.817
RRIM 600	NS	170.56	638.89	468.33	0.733	3.751
	S	468.00	341.45	170.89	0.500	0.730
Tjir1	NS	195.22	653.11	457.89	0.701	3.353
	S	561.33	287.00	91.78	0.320	0.511
MT 41	NS	214.44	781.11	566.67	0.725	3.669
	S	619.11	376.44	162.00	0.430	0.608
MT 76	NS	170.11	713.33	543.22	0.761	4.196
	S	629.00	254.44	84.33	0.331	0.405
MT 66	NS	164.78	630.89	467.11	0.739	3.836
	S	560.67	235.00	70.22	0.299	0.419
MT 938	NS	201.56	677.33	475.78	0.709	3.372
	S	558.78	320.11	118.55	0.370	0.573
AC 650	NS	190.33	744.56	554.22	0.744	3.91
	S	573.89	361.00	170.67	0.473	0.629
AC 652	NS	202.44	820.11	617.67	0.753	4.067
	S	616.33	406.22	203.78	0.502	0.659
RRII 105	NS	184.89	623.45	438.56	0.702	3.385
	S	417.56	390.78	205.89	0.527	0.936
AC 728	NS	213.56	695.22	481.67	0.693	3.258
	S	593.67	315.11	101.55	0.322	0.531
Mean	NS	194.67	719.43	524.84	0.728	3.717
	S	556.98	357.11	162.44	0.438	0.653

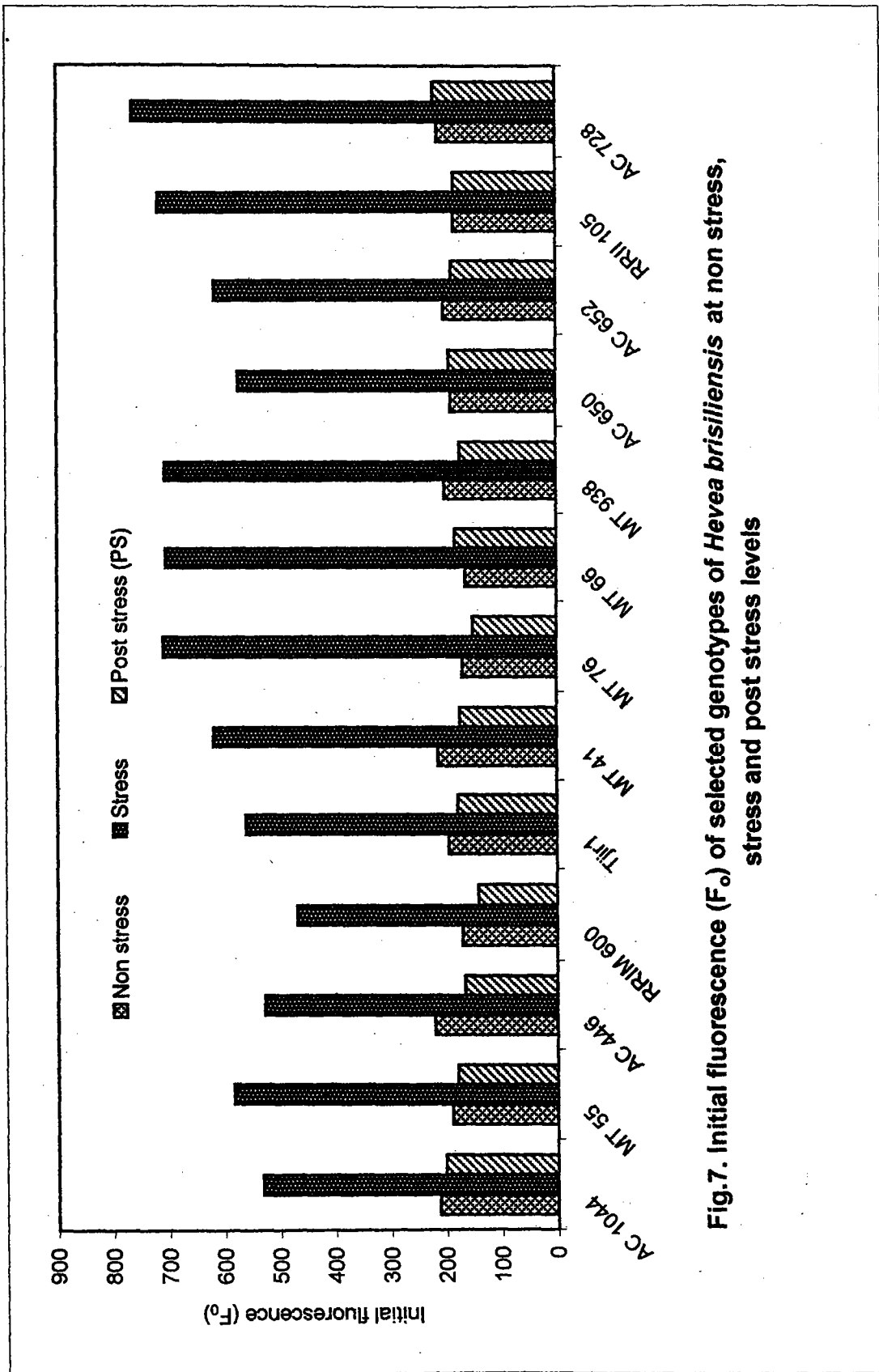


Fig.7. Initial fluorescence (F₀) of selected genotypes of *Hevea brasiliensis* at non stress, stress and post stress levels

Table 16. Result of Factorial CRD for initial fluorescence (F_o) of *Hevea* genotypes during non stress (NS) and stress (S) periods

Genotype	Non stress (NS)	Stress (S)	Mean	Conclusion	
AC 1044	212.67	531.78	372.22	SE plot ⁻¹	1.914
MT 55	189.33	583.78	386.56	Gen. Mean	408.17
AC 446	220.78	526.89	373.83	CV %	2.93
RRIM 600	170.56	468.00	319.28	Variance ratio	
Tjirl	195.22	561.33	378.28	Genotype	85.96**
MT 41	214.44	619.11	416.78	NS vs S	24875.18**
MT 76	170.11	709.00	439.56	G x NS vs S	94.37**
MT 66	164.78	704.67	434.72	CD (P=0.05)	
MT 938	201.56	705.78	453.67	Genotype	11.58
AC 650	190.33	573.89	382.11	NS vs S	4.542
AC 652	202.44	616.33	409.39	Interact	16.38
RRII 105	184.89	717.56	451.22		
AC 728	213.56	763.67	488.61		
Mean	194.67	621.68			

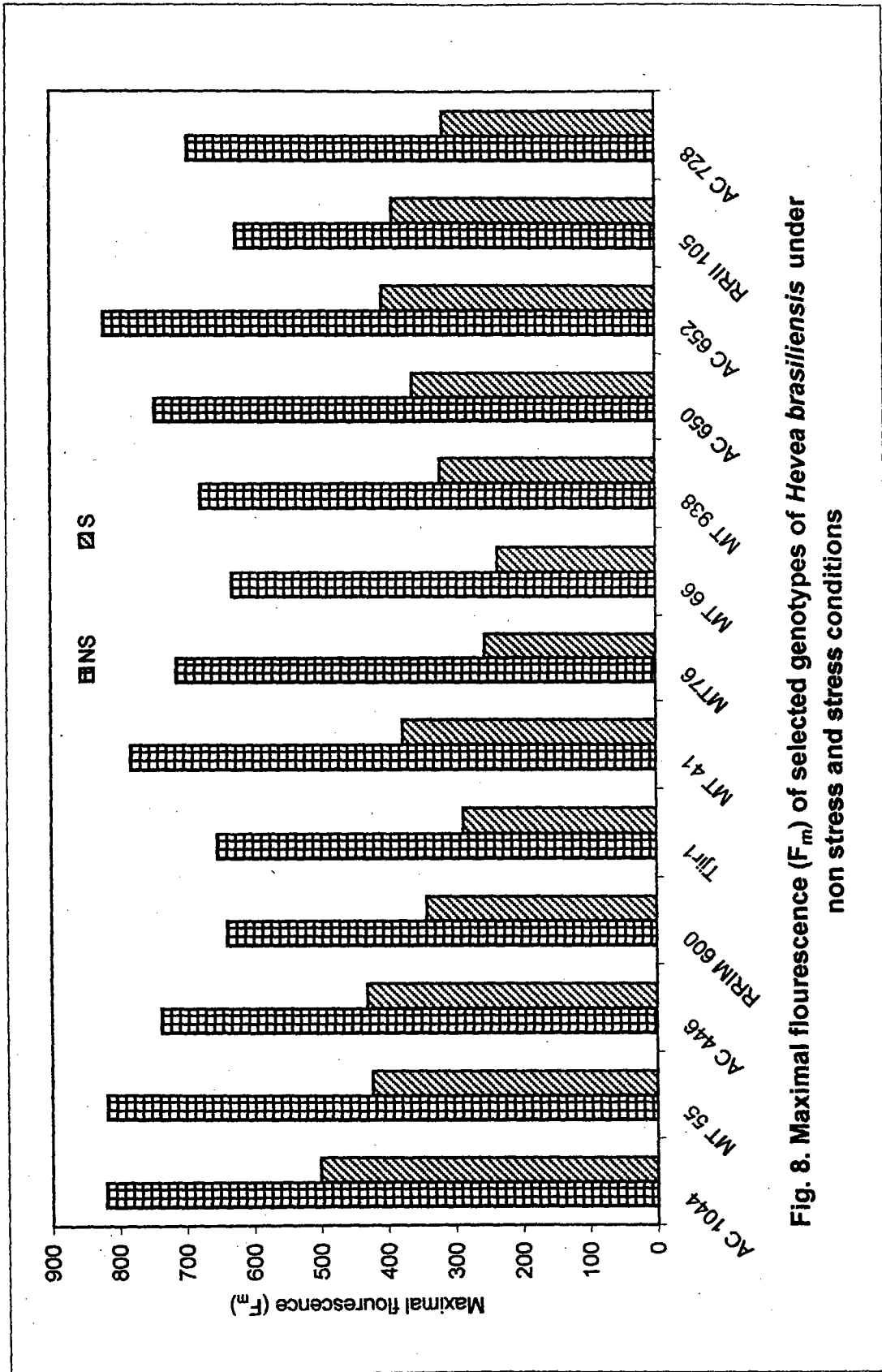


Fig. 8. Maximal fluorescence (F_m) of selected genotypes of *Hevea brasiliensis* under non stress and stress conditions

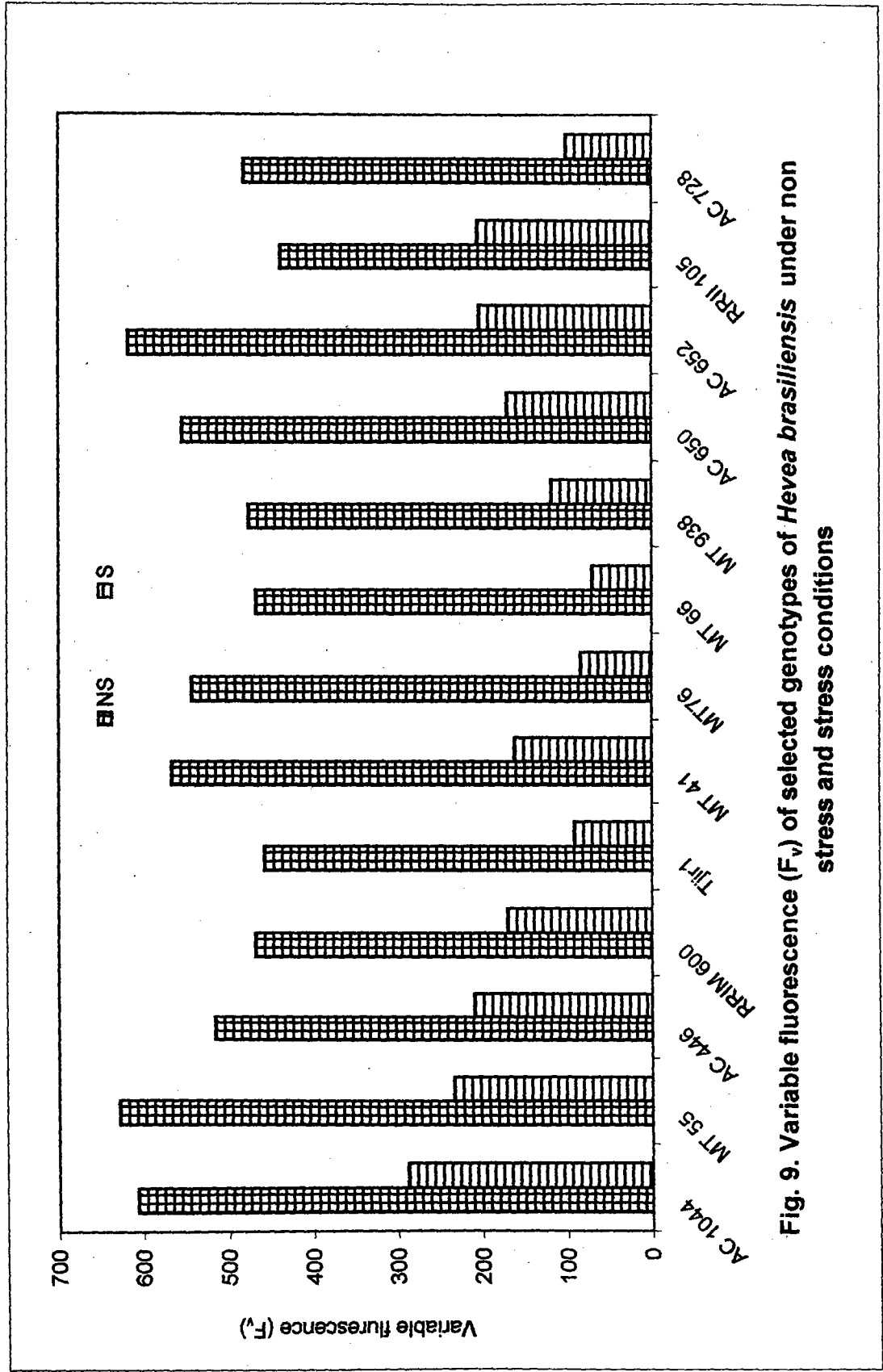


Fig. 9. Variable fluorescence (F_v) of selected genotypes of *Hevea brasiliensis* under non stress and stress conditions

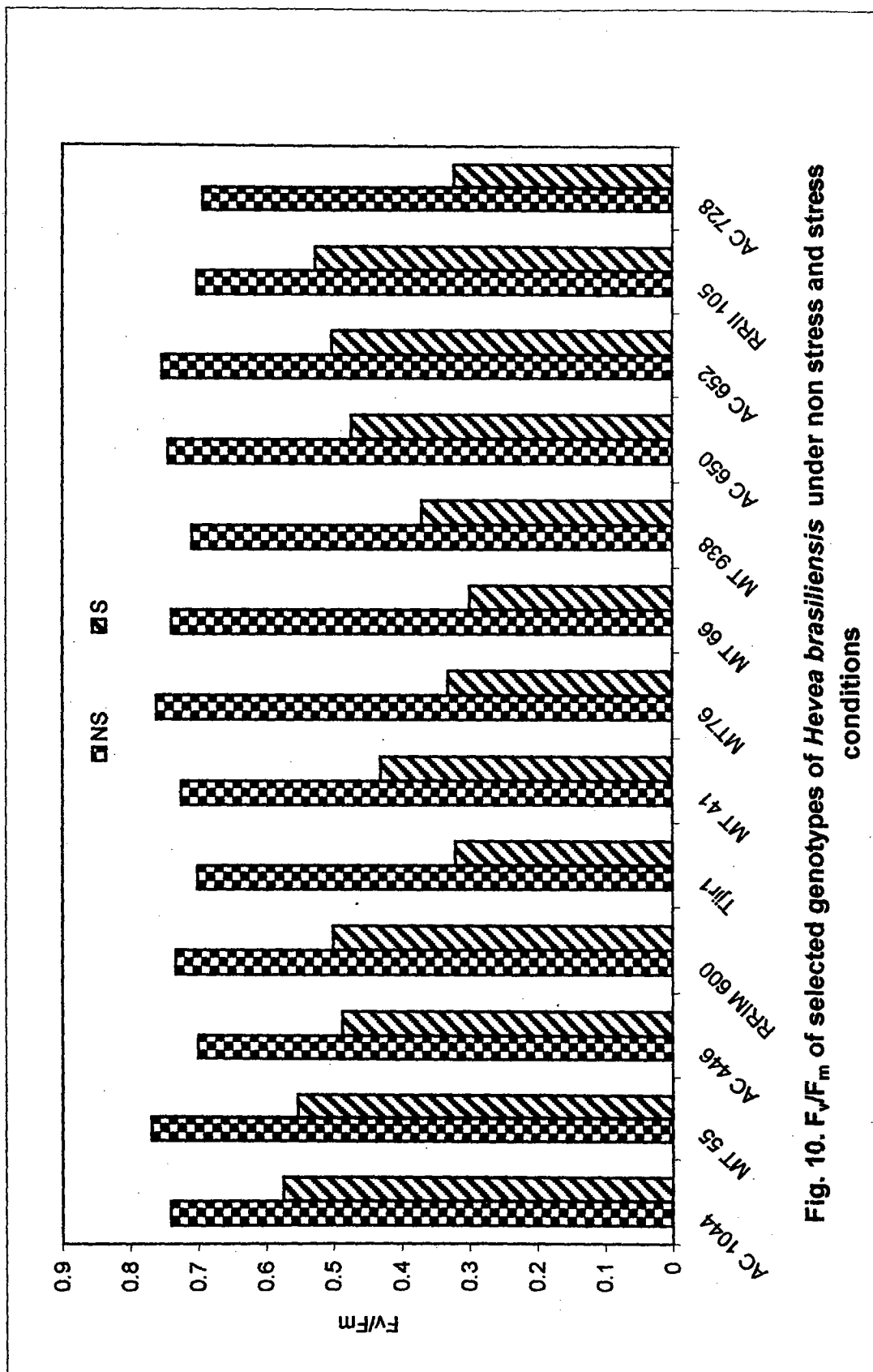


Fig. 10. F_v/F_m of selected genotypes of *Hevea brasiliensis* under non stress and stress conditions

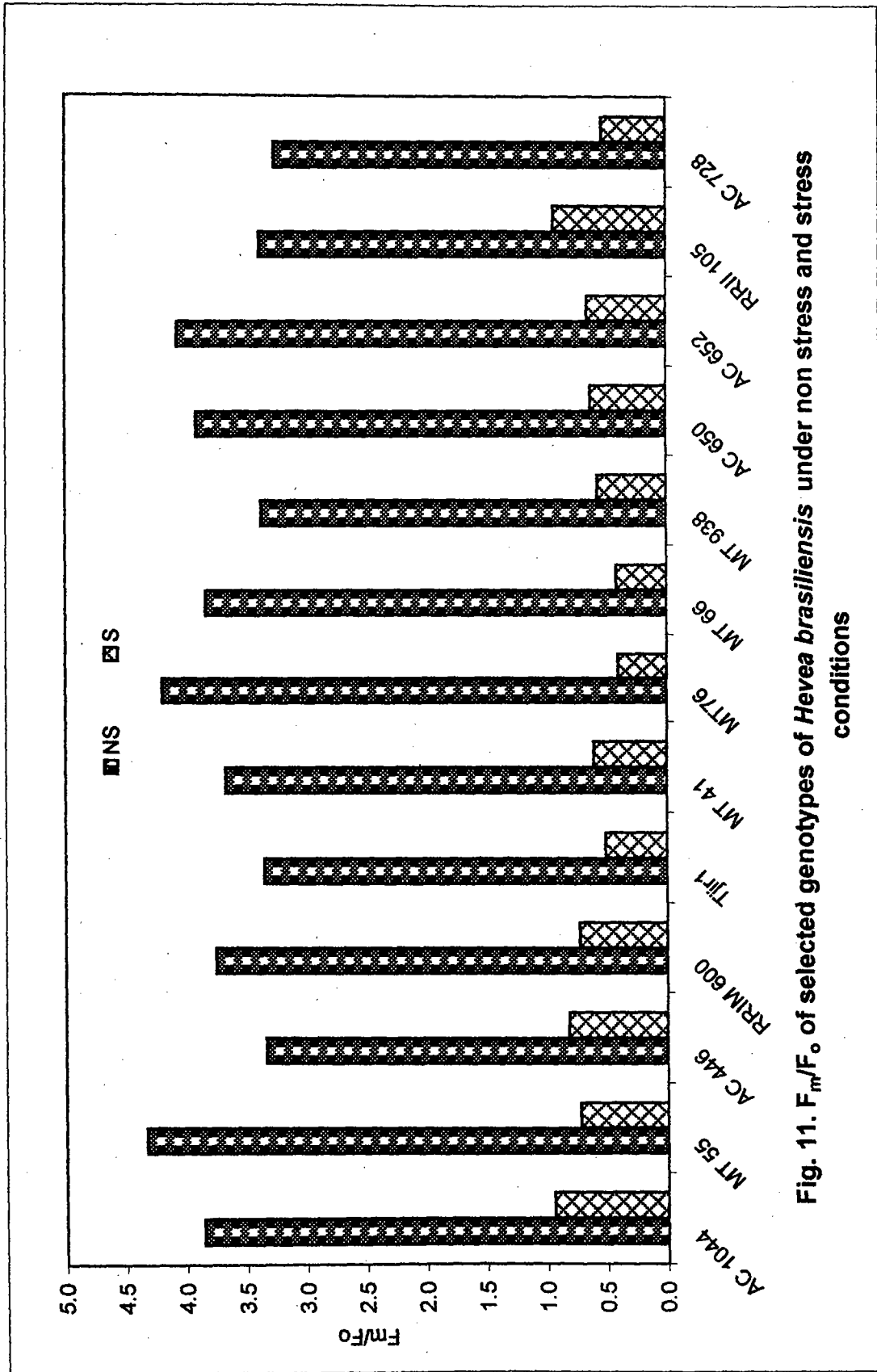


Fig. 11. F_m/F_o of selected genotypes of *Hevea brasiliensis* under non stress and stress conditions

between the genotypes, between NS vs S periods and genotype x NS vs S interaction effect.

4.3 Genetic variability for drought related morphological parameters

4.3.1 Field experiment

4.3.1.1 External appearance

The genotypic performance for various morphological parameters recorded are presented in Table 17. Plant height varied from 99.22 - 147.06 cm with a mean height of 118.31 cm. The genotypic difference was significant at 5 per cent level. The tallest genotype was MT 55, whereas the shortest was AC 652. The clones RRIM 600 and Tjir1 had similar heights, whereas clone RRII 105 was taller than these two clones.

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 12.47 mm. Though there was numerical difference in basal diameter between accessions the difference was not significant.

Number of flushes observed among the wild genotypes exhibited significant genotypic difference at 5 per cent level of significance. The mean number of flushes was 2.64 with a range of 1.78 - 3.81. The minimum number of flushes was seen in the genotype AC 652, whereas the genotype MT 41 possessed the maximum number of flushes, followed by the genotype MT 55. The number of flushes in the clones RRIM 600, Tjir1 and RRII 105 were comparable.

Total number of leaves present among the genotypes studied varied from 33.45 to 99.72 and the mean number of leaves computed was 63.88. The highest number of leaves was possessed by the genotype MT 41 followed by AC 650, whereas the lowest number of leaves was present in the genotype AC 652 followed by MT 66. Analysis of data indicated significant genotypic

Table 17. Morphological parameters of selected genotypes of *Hevea brasiliensis* at juvenile stage

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 ^o	11.14 ^{ab}	2.08 ^{cd}	49.00 ^{de}	28.05 ^{ab}	84.42 ^a	0.005 ^a
MT 55	147.06 ^a	13.23 ^{ab}	3.06 ^{abc}	90.89 ^{abo}	28.77 ^{ab}	87.05 ^a	0.006 ^a
AC 446	115.77 ^{abo}	13.49 ^{ab}	2.83 ^{abcd}	48.47 ^{de}	35.26 ^{ab}	86.84 ^a	0.005 ^a
RRIM 600	104.95 ^o	11.61 ^{ab}	2.28 ^{bcd}	51.44 ^{de}	22.67 ^b	58.82 ^b	0.006 ^a
Tjir1	104.90 ^o	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}	3.00 ^{abc}	77.22 ^{abcd}	28.59 ^{ab}	60.85 ^b	0.006 ^a
MT 66	99.75 ^o	13.63 ^{ab}	2.69 ^{bcd}	48.25 ^{de}	19.57 ^b	81.56 ^a	0.007 ^a
MT 938	130.67 ^{abc}	11.50 ^{ab}	2.17 ^{cd}	60.33 ^{bode}	43.92 ^a	84.80 ^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 ^o	10.14 ^b	1.78 ^d	33.45 ^e	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}	13.10 ^{ab}	2.33 ^{bcd}	55.44 ^{ode}	24.47 ^b	90.78 ^a	0.005 ^a
Mean	118.31	12.47	2.64	63.88	27.07	77.13	0.006

Any two means having a common letter are not significantly different

difference for the total number of leaves present in these genotypes. Clones RRIM 600 and RRII 105 were having similar leaf number, whereas the total leaf number of Tjir1 was more than these two clones.

When the interflush distance was studied, there was no significant genotypic difference but the character varied in each genotype. The mean interflush distance recorded was 27.07 cm and the range was 19.57 - 43.92 cm. The lowest interflush distance was observed in the wild genotype MT 66 and the highest was in MT 938. The clones RRIM 600, Tjir1 and RRII 105 were on par.

Single leaflet area exhibited significant genotypic difference. Wild genotypes in general recorded higher leaf area than the cultivated clones. The highest leaflet area recorded was 90.78 cm² in the genotype AC 728 and the lowest was 59.3 cm² in the clone Tjir1. The clones RRIM 600 and RRII 105 were also on par with Tjir1. The mean leaflet area recorded was 76.78 cm².

Specific leaf weight did not show any significant difference among the genotypes. However, the genotypes expressed slight variation among each other, where the range was 0.005 - 0.007 g cm⁻² with a mean of 0.006 g cm⁻². SLW of Tjir1 and RRII 105 was 0.007 g cm⁻² whereas the same in RRIM 600 was 0.006 g cm⁻².

4.3.2 Glass house experiment

4.3.2.1 Effect of water stress on basal diameter

When the effect of water stress on basal diameter was studied (Table 18), there was significant genotypic effect for the difference in basal diameter under water stress. In most of the wild genotypes and in control clones, water stress reduced the basal diameter. Girth reduction in *Hevea* clones as a result of water stress has been reported earlier and the result obtained in the present study confirms the earlier reports. When the difference in basal diameter was expressed

Table 18. Influence of water stress on basal diameter of selected genotypes of *Hevea brasiliensis* at juvenile stage

Genotype	Basal diameter (mm)						Growth depression (%)
	Non stress	Stress	Increase (%)	Non stress-1	Non stress-2	Increase (%)	
AC 1044	7.20	6.999	-6.97 ^f	7.20	7.80	8.33	15.297
MT 55	7.13	6.987	-2.00 ^d	8.07	8.67	6.92	8.920
AC 446	7.63	7.433	-2.60 ^{de}	8.50	8.77	3.08	5.677
RRIM 600	7.09	6.977	-1.59 ^d	7.57	8.10	7.00	8.587
Tjir1	6.68	6.570	-1.66 ^d	6.50	7.53	15.85	17.513
MT 41	7.19	7.478	4.03 ^a	7.17	7.97	11.16	7.127
MT76	7.30	7.767	0.65 ^{bc}	7.25	7.96	8.92	8.273
MT 66	6.24	6.470	0.37 ^e	6.67	7.50	12.44	12.070
MT 938	7.90	8.010	1.36 ^{bc}	7.40	8.00	7.50	6.137
AC 650	7.68	7.534	-1.95 ^d	7.57	8.27	9.25	11.203
AC 652	7.43	7.570	1.96 ^b	7.10	7.43	4.44	2.483
RRII 105	7.57	7.290	-3.66 ^e	6.97	7.50	7.60	11.257
AC 728	7.61	7.389	-2.93 ^{de}	7.80	8.37	7.31	10.243
Mean	7.28	6.685	-1.15	7.37	7.99	8.45	9.599

Any two means having a common letter are not significantly different

as increase or decrease percentage, the highest reduction percentage was noticed in AC 1044, followed by AC 728 and the lowest in the genotype MT 41. The decrease in basal diameter was 1.58 per cent in RRIM 600, 1.66 per cent in Tjir1 and 3.68 per cent in RRII 105. The genotypes MT 76, MT 938, MT 66 and AC 652 expressed slight increase in basal diameter under water stress. Under non-stress condition, basal diameter had increased in all the genotypes and the maximum increase rate was noticed in Tjir1, followed by MT 66. In the clones RRIM 600 and RRII 105, the percentage increase of basal diameter was on par. Growth depression in terms of basal diameter was compared under non-stress and stress conditions, where the maximum growth depression was noticed in the clone Tjir1, followed by AC 1044. The growth depression observed in RRII 105 was 11 per cent which was more than that in RRIM 600 where the depression was 9 per cent. AC 652 expressed the lowest growth reduction of 2.48 per cent and the next genotype with less growth reduction was AC 446.

4.3.2.2 Effect of water stress on dry matter production.

The dry weight of the scion portion under non-stress and stress conditions were considered for calculating the dry matter stress tolerance index (DMSI) and the results are shown in Table 19. Under non stress condition, almost all the genotypes produced more or less similar quantity of dry matter. The range varied from 17.93 - 28.1 g with a mean dry matter production of 22.63 g. Among the wild genotypes, the highest dry matter production was obtained in the genotype MT 938 followed by AC 1044 and the lowest was in AC 652. All the 3 standard clones had comparable dry matter production which was similar to the mean value.

However, under stress condition the wild genotypes as well as the control clones reacted differently. The dry matter production was considerably reduced in all the genotypes. The range was between 6.97 - 19.83 g with a mean

Table 19. Dry matter stress tolerance index (DMSI) of selected genotypes of *Hevea brasiliensis* at juvenile stage

Genotype	Non stress (g)	Stress (g)	DMSI
AC 1044	26.10 ^{ab}	12.33 ^b	53.86 ^{bc}
MT 55	26.01 ^{ab}	9.27 ^b	63.99 ^{ab}
AC 446	21.70 ^{ab}	14.01 ^{ab}	35.79 ^{def}
RRIM 600	23.93 ^{ab}	10.57 ^b	56.25 ^b
Tjir1	24.62 ^{ab}	6.97 ^b	71.70 ^a
MT 41	19.02 ^b	13.00 ^b	31.80 ^{def}
MT76	23.37 ^{ab}	13.39 ^b	43.65 ^{cd}
MT 66	21.63 ^{ab}	7.67 ^b	64.51 ^{ab}
MT 938	28.10 ^a	19.83 ^a	30.49 ^{ef}
AC 650	18.87 ^b	7.50 ^b	60.50 ^{ab}
AC 652	17.93 ^b	13.13 ^b	27.85 ^f
RRII 105	21.33 ^{ab}	12.87 ^b	40.34 ^{de}
AC 728	21.61 ^{ab}	12.47 ^b	43.23 ^{cd}
Mean	22.63	11.77	47.99

Any two means having a common letter are not significantly different

value of 11.77 g. Though there was significant reduction in dry matter in all the clones, the reduction in Tjir1 was so drastic. Analysis of data indicated significant genotypic difference.

The range in terms of DMSI varied between 27.85 - 71.7 with a mean of 47.99. The highest stress tolerance in terms of DMSI was noticed in the wild genotype AC 652 followed by MT 41 where the values were 27.85 and 31.8 respectively, whereas the lowest was noticed in the genotype MT 66 (63.99) followed by MT 55 (64.51). The tolerance index in the control clones RRIM 600 and RRII 105 were 56.25 and 40.34 and that of Tjir1 was 71.7. There was significant genotypic difference for the DMSI worked out.

4.4 Genetic variability for drought related biochemical parameters

The variability present among the genotypes for the epicuticular wax content (ECW), total chlorophyll content and chlorophyll reduction percentage as a result of heat treatment is shown in Table 20. The total wax content among the genotypes varied from 19.31 - 44.04 $\mu\text{g cm}^{-2}$ with a mean of 28.07 $\mu\text{g cm}^{-2}$. Clone RRII 105 recorded the highest ECW content and the lowest was in MT 938. Among the wild genotypes, MT 41 and AC 652 recorded relatively high ECW content. The ECW content of the drought susceptible clone Tjir1 (31.94 $\mu\text{g cm}^{-2}$) was more than that in the drought tolerant clone RRIM 600 (26.76 $\mu\text{g cm}^{-2}$). Scanning electron micrographs of the adaxial leaf surface of RRIM 600, RRII 105, Tjir1 and MT 41 showing the variation in epicuticular wax content are given in Plates 1 to 4. Analysis of the data indicated significant genotypic difference in the ECW content of these genotypes.

The total chlorophyll content also varied from 1.75 - 4.65 mg cm^{-2} with a mean of 2.8 mg cm^{-2} . The highest was recorded in the clone Tjir1, followed by

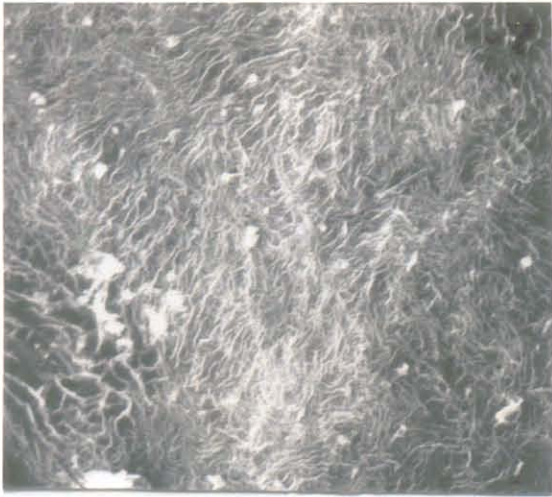
Plates 1-4. Scanning electron micrographs showing the variation in leaf epicuticular wax content in different *Hevea* clones and in wild genotype



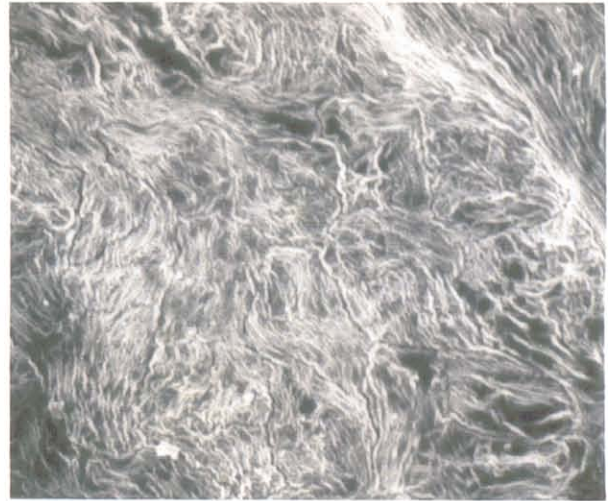
Tjir1



RR11 105



RR1M 600



MT 41

Table 20. Variation in leaf epicuticular wax (ECW), chlorophyll content and chlorophyll reduction per cent in the young plants of different genotypes of *Hevea brasiliensis*

Genotypes	ECW ($\mu\text{g cm}^{-2}$)	Chlorophyll content (mg cm^{-2})	Chlorophyll reduction (%)
AC 1044	24.91 ^{cd}	2.98 ^a	2.02 ^{ef}
MT 55	25.55 ^{cd}	2.93 ^a	15.19 ^a
AC 446	25.24 ^{cd}	1.75 ^a	1.20 ^f
RRIM 600	26.76 ^{cd}	3.38 ^a	0.92 ^f
Tjir1	31.94 ^{bc}	4.65 ^b	11.29 ^b
MT 41	39.12 ^{ab}	3.63 ^a	4.64 ^e
MT 76	26.24 ^{cd}	2.90 ^a	12.66 ^{ab}
MT 66	22.32 ^{cd}	3.47 ^a	7.59 ^{cd}
MT 938	19.31 ^d	1.84 ^a	1.91 ^{ef}
AC 650	25.05 ^{cd}	1.79 ^a	12.77 ^{ab}
AC 652	28.89 ^{cd}	2.45 ^a	4.88 ^{de}
RRII 105	44.04 ^a	2.00 ^a	1.25 ^f
AC 728	25.47 ^{cd}	2.64 ^a	8.09 ^c
Mean	28.07	2.80	6.49

Any two means having a common letter are not significantly different

MT 41, MT 66 and RRIM 600. Lowest chlorophyll content was observed in the genotype AC 446 followed by AC 650. There was significant genotypic difference for the total chlorophyll content studied among the selected genotypes.

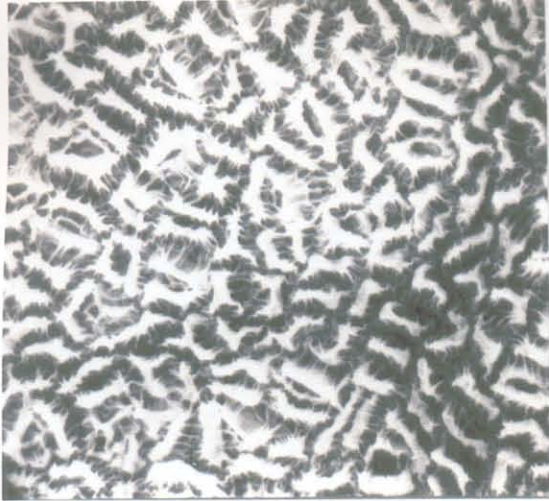
Chlorophyll reduction percentage after heat treatment was worked out in each genotype in order to assess the variability in chlorophyll stability in the genotypes when they are exposed to heat stress. The percentage reduction varied from 0.92 - 15.19 with a mean of 6.49. The highest chlorophyll reduction was noticed in the wild genotype MT 55 followed by AC 650 whereas the lowest was in the control clone RRIM 600, followed by AC 446 and RRII 105. The chlorophyll reduction percentage as a result of heat treatment was fairly high in the genotype MT 76 and in the clone Tjir1. There was significant genotypic difference for chlorophyll reduction among these wild genotypes and control clones.

4.5 Genetic variability for drought related anatomical parameters

4.5.1 Leaf anatomical characters

The variability among the selected genotypes for the various leaf anatomical characters studied is presented in Table 21. The number of stomata per mm² of leaf lamina varied from 276.99 - 481.48, with a mean stomatal density of 376.19. The highest number of stomata was seen in the genotype MT 66 followed by AC 1044 and the lowest in the genotype AC 446. The stomatal density of control clones RRIM 600, Tjir1 and RRII 105 were 356.67, 446.62, and 427.01 respectively. Scanning electron micrographs of the abaxial leaf surface of RRIM 600, RRII 105, Tjir1 and AC 446 showing the variation in stomatal density and size are given in Plates 5 to 8. In majority of the wild genotypes the stomatal number per unit area of leaf lamina was lesser than the mean value. There was significant genotypic difference for the number of stomata per unit area.

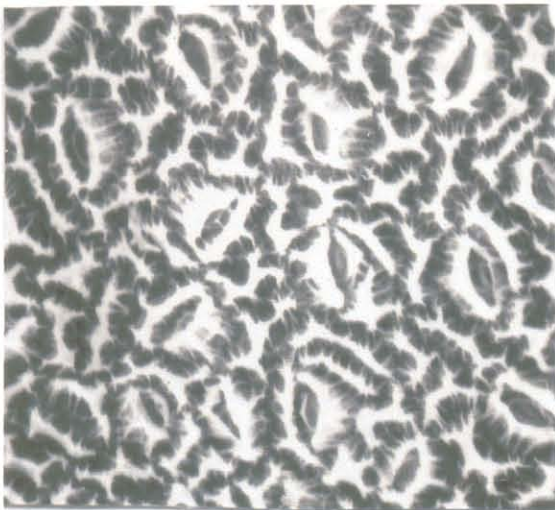
Plates 5-8. Scanning electron micrographs showing the variation in stomatal density and size in different *Hevea* clones and in wild genotype



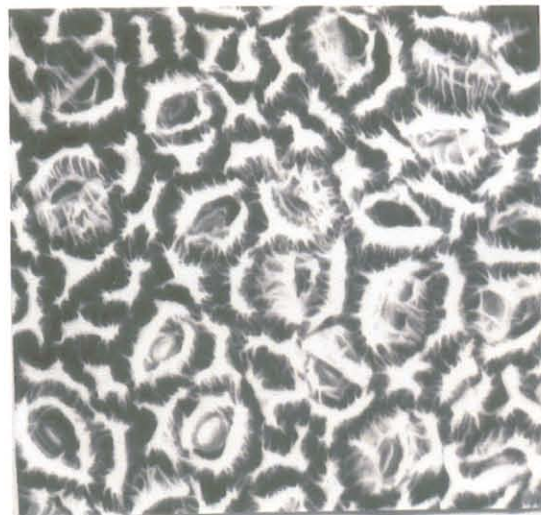
Tjir1



RRIM 600



RR11 105



AC 446

Table 21. Variability for leaf anatomical characters of selected genotypes of *Hevea brasiliensis*

Genotype	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 ^{bedo}	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 ^{bede}	371.00 ^{bed}	47.73 ^{efg}	84.95 ^f	30.22 ^{bed}
RRIM 600	356.67 ^{abcd}	123.77 ^{abc}	352.20 ^{defg}	51.36 ^{cdef}	105.79 ^{abc}	35.24 ^a
Tjiri1	446.62 ^{ab}	129.83 ^a	406.80 ^{ab}	64.65 ^a	114.52 ^a	24.29 ^f
MT 41	333.33 ^{bed}	104.23 ^g	360.73 ^{cde}	44.14 ^{fg}	82.67 ^f	33.51 ^{abo}
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 ^{abo}
AC 650	331.16 ^{bed}	114.43 ^{cdef}	355.18 ^{def}	50.43 ^{defg}	95.65 ^{bedef}	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}
Mean	376.19	119.03	361.94	52.55	98.18	31.30

Any two means having a common letter are not significantly different

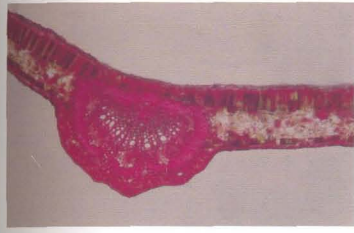
Cross sectional view of the leaf lamina of the 13 genotypes depicting the characters leaf thickness, midrib diameter, palisade tissue thickness, mesophyll tissue thickness and palisade cell number per unit length are shown in Plates 9 to 21. Leaf thickness of the genotypes studied varied from 104.23 - 129.83 μm and the mean was 119.03 μm . The highest leaf thickness value was noticed in the clone Tjir1 followed by the wild genotypes MT 76 and AC 652. Leaf thickness of RRIM 600 and RRII 105 were 123.77 and 127.27 μm respectively. Genotypes AC 1044, MT 76, MT 938 and AC 652 had leaf thickness higher than the mean value. Analysis of data indicated significant genotypic difference for this character.

The range of midrib diameter was 310.67 - 438.9 μm with a mean of 361.94 μm . The highest midrib diameter was recorded in the wild genotype AC 1044 followed by MT 66 and the lowest was in MT 55. All the control clones had comparatively higher midrib diameter. The wild genotypes AC 1044, AC 446, MT 41 and MT 66 had relatively higher midrib diameter. Analysis of data indicated significant genotypic difference.

Thickness of palisade tissue was in the range of 42.42 - 64.65 μm , with a mean value of 52.55 μm . Among the wild genotypes the highest palisade tissue thickness was noticed in AC 652 followed by MT 76 whereas the lowest was in MT 938 and in MT 41. The control clones RRIM 600, RRII 105 and Tjir1 were having comparatively good palisade tissue thickness and in majority of the wild genotypes the thickness of palisade tissue was lower than the mean value. However, the palisade tissue thickness of the genotypes AC 1044, MT 76 and AC 652 was greater than the mean value. Here also, the genotypic difference was significant.

The mesophyll tissue thickness measured was in the range of 84.95 - 114.52 μm and the mean mesophyll tissue thickness was 98.18 μm . The

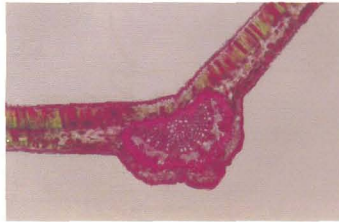
Plates 9-13. Cross sectional view of leaf lamina of various wild genotypes of *Hevea brasiliensis*



AC 650



AC 652



AC 728



MT 938

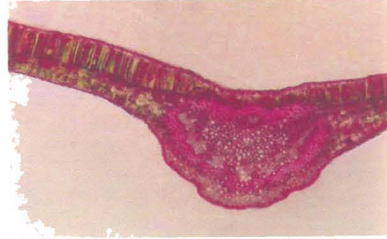


MT 66

Plates 14-18. Cross sectional view of leaf lamina of various wild genotypes of *Hevea brasiliensis*



MT 41



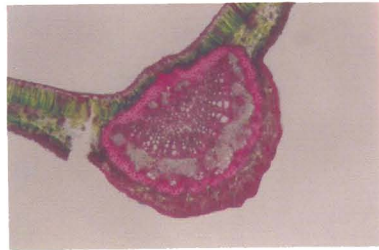
MT 76



AC 446

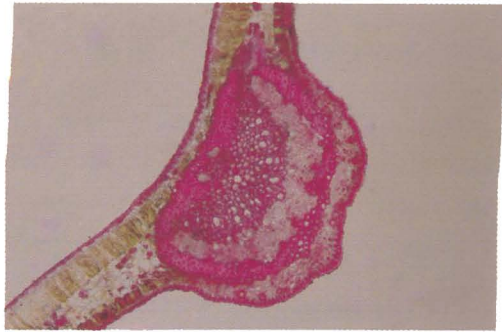


MT 55

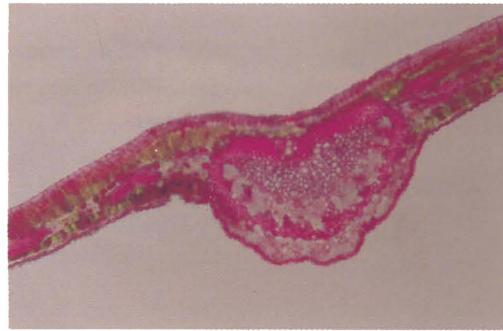


AC 1044

**Plates 19-21. Cross sectional view of leaf lamina of control clones of
*Hevea brasiliensis***



RRH 105



RRIM 600



Tjir1

genotype AC 652 recorded the highest mesophyll tissue thickness of 109.79 μm among the wild genotypes and the lowest was in AC 446. As in the case of other leaf structural parameters, the mesophyll tissue thickness among the control clones was higher than the mean value. The genotypes AC 1044, MT 76, MT 938 and AC 652 possessed higher mesophyll tissue thickness than the mean. Here also, the genotypes exhibited significant genotypic difference.

Palisade cell number per unit length recorded varied from 24.29 - 35.24 with a mean of 31.3. Among the wild genotypes, the maximum palisade cell number was seen in MT 66 followed by AC 1044 where the number was 34.59 and 34.26 respectively and the lowest was recorded in AC 728 with a cell number of 26.04. Among the wild genotypes, 50 per cent recorded higher values than the mean. The control clones RRIM 600 and RRII 105 recorded higher number of palisade cells than the mean value, whereas the same in Tjir1 was far below the mean value. Analysis of data indicated significant genotypic difference for this character also.

4.5.2 Bark anatomical characters

Data on total bark thickness, proportion of soft bast region, total latex vessel rows, and proportion of latex vessel rows in the soft bast are represented in Table 22. The total bark thickness varied from 1.277 - 1.623 mm with a mean of 1.398 mm. The highest was in the wild genotype MT 41 followed by MT 66 and the lowest bark thickness was in the genotype AC 650. There was no significant genotypic difference for this character, and majority of the wild genotypes and the selected control clones were on par.

Similarly, soft bast thickness also did not show any significant genotypic difference. The range observed was 0.57 - 0.77 mm with a mean value of 0.66 mm. The soft bast thickness was high in the wild genotypes AC 446, AC 1044, and MT 66 while the genotypes MT 55 and MT 938 recorded lower

Table 22. Variability for bark anatomical characters of selected genotypes of *Hevea brasiliensis* at juvenile stage

Genotype	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
Tjiri1	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
RRJI 105	1.347 ^{ab}	0.67 ^{ab}	49.61 ^{abc}	3.00 ^a	1.89 ^a	63.00 ^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

soft bast thickness. The clones RRIM 600 and RRII 105 had comparable soft bast thickness, whereas in Tjir1, the value was higher.

The range in the proportion of soft bast thickness was 38.93 - 56.53 per cent and the mean was 47.67 per cent. Proportion of soft bast was highest in AC 446 and in AC 1044 and the lowest was in MT 55. All the 3 control clones had more or less similar soft bast proportion. The genotypes AC 446 and MT 938 differed significantly for their soft bast proportion.

In the case of total number of latex vessel rows (LVR) the variation observed among the genotypes was in the range of 1.33 - 3.33 with a mean of 2.76. MT 41 recorded the highest LVR, while the lowest was in MT 938. Among the wild genotypes, MT 41 had a higher number of LVR than the three controls while the value was comparable in AC 1044, AC 446 and in AC 650. There was no significant genotypic difference for LVR except for MT 938.

The range in the number of LVR in the soft bast was 1.33 – 2.33 and the mean was 1.95. No significant genotypic difference was noticed for number of LVR in the soft bast. The proportion of LVR in the soft bast region varied from 63 - 100 per cent with a mean of 72.41 per cent. Among the wild accessions, AC 1044 and MT 41 had the lowest proportion whereas the highest was in MT 938. Significant genotypic difference for this character was noticed only in MT 938. Compared to RRIM 600 and Tjir1 (66.67%), the proportion was low in RRII 105 (63%).

4.6 Genetic parameters

Estimates of variability and the genetic parameters were worked out for certain important morphological (plant height, basal diameter, number of flushes, number of leaves, single leaflet area), anatomical (number of stomata, leaf thickness, palisade tissue thickness, palisade cell number per unit length, bark

Table 23. Phenotypic and genotypic coefficients of variation (PCV, GCV), heritability (H^2) and genetic advance (GA) of selected parameters of *Hevea* genotypes

Characters	PCV	GCV	H^2	GA as % of mean
Plant height	18.69	12.86	47.4	21.57
Basal diameter	17.88	6.17	43.38	21.27
No. of flushes	27.76	17.70	40.70	14.38
No of leaves	40.54	28.23	48.50	25.86
Single leaflet area	23.63	15.66	43.90	16.42
No. of stomata	22.91	12.13	28.00	49.80
Leaf thickness	8.24	6.92	70.60	14.26
Palisade tissue thickness	14.69	12.15	68.30	10.87
Palisade number/unit length	12.06	10.16	70.90	5.51
Bark thickness	11.17	10.89	92.00	31.62
Total no of L.V.R	26.28	13.26	72.37	58.39
ECW	28.09	21.80	60.60	9.78
Chlorophyll content	33.43	28.97	75.00	11.45
Stomatal conductance	46.11	41.92	83.45	29.55
Transpiration rate	38.31	36.33	88.50	14.58

thickness, total number of LVR), biochemical (ECW and chlorophyll contents) and physiological (stomatal conductance, transpiration rate) characters. The results are shown in Table 23.

Phenotypic coefficient of variation (PCV) was high for most of the morphological characters considered. Total number of leaves exhibited a fairly high PCV (40.54) followed by number of flushes (27.76). All the leaf anatomical characters had low to medium PCV with the highest (22.91) for number of stomata. Among the bark structural traits, total number of LVR had a high value of 26.28 while it was 11.17 for bark thickness. Both the physiological parameters - stomatal conductance and transpiration rate had higher PCV of 46.11 and 38.31 respectively. The PCV exhibited by ECW content was 28.09 and it was 33.43 for chlorophyll content.

Among the morphological characters, total number of leaves exhibited maximum genotypic coefficient of variation (GCV) of 28.23 followed by number of flushes and single leaflet area where the GCV was 17.7 and 15.66 respectively. Among the leaf structural characters studied, palisade tissue thickness exhibited maximum GCV of 12.15 closely followed by number of stomata on the leaf surface (12.13). Bark thickness and total number of L.V.R. exhibited a GCV of 10.89 and 13.26 respectively. The GCV of wax content was 21.8 and it was 28.97 for chlorophyll content. The physiological parameters namely stomatal conductance and transpiration rate exhibited a fairly good GCV of 41.92 and 36.33 respectively.

Estimates of heritability in the broad sense (H^2) indicated medium to high heritability for all the characters considered. As expected all the morphological characters exhibited medium heritability, whereas all the leaf and bark structural characters as well as physiological and biochemical characters exhibited a high H^2 . The value was comparable for all the leaf

structural characters studied except for number of stomata, which had a low H^2 (28.0). For bark structural characters, the H^2 for bark thickness was fairly high (92.0) compared to that of total number of LVR (72.37). H^2 for stomatal conductance and transpiration rate were 83.45 and 88.50 respectively. Among the biochemical characters, ECW content exhibited H^2 of 60.60 whereas that of chlorophyll content was fairly high (75.0).

Genetic advance (GA) as percentage of mean was estimated, which showed low to medium values for almost all the characters studied, except for total number of LVR, where a high GA of 58.39 was estimated, which was followed by number of stomata (49.8).

4.7 Character associations

The phenotypic and genotypic correlation coefficients worked out for various characters are shown in Table 24 and Table 25. Basal diameter showed negligible phenotypic correlation and very high positive genotypic correlation with single leaflet area and with plant height, the phenotypic correlation was high whereas genotypic correlation was negative. Genotypic and phenotypic correlation of basal diameter with number of whorls and number of leaves are positive and very high. Similarly both phenotypic and genotypic correlation coefficients of plant height and number of leaves with number of whorls were very high and positive. Number of leaves and interflush distance also showed very high and positive correlation coefficients with plant height. Leaf thickness showed a high negative genotypic correlation and negative phenotypic correlation with single leaflet area, while with palisade tissue thickness both correlation coefficients were high and positive. Bark thickness with specific leaf weight showed only negligible correlation coefficients. Total number of latex vessel rows had very high positive genotypic correlation coefficient and negligible phenotypic correlation coefficient with number of flushes and with basal diameter, the

Table 24. Character association between morphological and structural parameters of *Hevea brasiliensis* at juvenile stage

Basal diameter Vs	- plant height	P 0.591	Plant height Vs	- No of leaves	P 0.732
	- No of flushes	G -0.986		- Inter flush distance	G 0.893
	- No of leaves	P 0.652			P 0.352
	-single leaflet area	G 0.147	Leaf thickness Vs		G 0.657
		P 0.525		- Single leaflet area	P -0.404
		G 1.220		- Palisade tissue thickness	G -0.723
No of whorls Vs		P 0.226			P 0.578
	- Plant height	G 0.881	Bark thickness Vs		G 0.881
	- No of leaves	P 0.659		- SLW	P 0.137
		G 0.746			G 0.264
		P 0.789	LVR Vs		
		G 0.978		- No of flushes	P 0.268
				- Basal diameter	G 0.702
					P 0.129
				- No of leaves	G 1.630
					P 0.177
					G 0.288

P = Phenotypic correlation
G = Genotypic correlation

Table 25. Character association between physiological and biochemical parameters of *Hevea brasiliensis* at juvenile stage

Stomatal conductance vs			
	- Transpiration rate	P	0.875
		G	1.027
	- Leaf temperature	P	-0.171
		G	-0.474
Fv/Fm vs			
	- Stomatal conductance	P	-0.505
		G	-0.525
	- Transpiration rate	P	-0.527
		G	-0.554
Epicuticular wax vs			
	- Leaf water potential	P	0.167
		G	1.587

P = Phenotypic correlation

G = Genotypic correlation

genotypic correlation was very high and positive and the phenotypic correlation was negligible. Number of leaves and LVR had negligible phenotypic and genotypic correlation coefficients.

Table 25 depict the character association of physiological and biochemical parameters. Transpiration rate showed very high positive genotypic and phenotypic correlation with stomatal conductance. F_v/F_m ratio of chlorophyll fluorescence showed high negative correlation coefficients with stomatal conductance and transpiration rate. Stomatal conductance showed negative genotypic correlation and negligible phenotypic correlation with leaf temperature. Soil water potential had high positive genotypic and phenotypic correlation with stomatal conductance and transpiration rate. Epicuticular wax content showed very high positive genotypic correlation and negligible phenotypic correlation with leaf water potential.

4.8 D^2 analysis

Genetic divergence existing in the population of wild genotypes was assessed in terms of "generalized group distance" using Mahalanobis D^2 analysis. Two separate D^2 analyses were done to determine the genetic distance between the 13 treatments, one for morphological and structural parameters and the second for physiological and biochemical parameters. The clustering of the genotypes was done by the Tocher's method of clustering. The result obtained is presented in Table 26. Based on morphological and structural characters, the 13 wild genotypes and control clones were grouped into 6 clusters. First cluster contained the genotypes MT 66 and AC 652 and the second one MT 55 and MT 938. The maximum number of genotypes viz., AC 446, RRIM 600, AC 650, RRII 105 and AC 728 were in the third cluster. Fourth and fifth cluster have only one genotype each, MT 41 and AC 1044 respectively while the last cluster included Tjir1 and MT 76. The average intracluster distance was 46.11.

Table 26. The distribution of *Hevea* genotypes into clusters by D² analysis

Clustering based on morphological and structural characters		Clustering based on physiological and biochemical characters	
Cluster no.	Genotypes	Cluster no.	Genotypes
1.	MT 66, AC 652	1.	AC 1044, MT 66, AC 652
2.	MT 55, MT 938	2.	MT 55, AC 650
3.	AC 446, RRIM 600, AC 650, RRII 105, AC 728	3.	AC 446, MT 41, MT 938
4.	MT 41	4.	MT 76
5.	AC 1044	5.	Tjir1, AC 728
6.	Tjir1, MT 76	6.	RRIM 600, RRII 105

Average intra cluster distance - 46.11

Average intra cluster distance - 0.81

Clustering based on physiological and biochemical characters also resulted in the same number of groups but the position of genotypes in the clusters was different. However, both clustering grouped the control clones RRIM 600 and RRII 105 into one cluster while the clone Tjir1 was included in another cluster. Average intracluster distance here was 30.81.

4.9 Selection of superior genotypes based on rank sums

The ranking of each genotype based on parametric relationships with drought tolerance are shown in Table 27. The rank sums varied from 65 to 121. The highest rank sum was obtained by the genotype MT 41, followed by MT 55 and AC 650. The lowest rank sum was shown by the genotype MT 66, closely followed by MT 938.

Table 27. Ranking of selected genotypes of *Hevea brasiliensis* based on parametric relationship with drought tolerance

GT	PH	BD	NL	SLA	GID	DMSI	SC	TR	F _v /F _m	ECW	CC	NS	PTT	SBP	SBL	CR	Rank sum
AC 1044	3	2	4	6	1	2	9	10	10	3	8	2	8	2	9	8	87
MT 55	10	7	8	9	4	3	8	8	9	7	7	4	4	8	5	1	102
AC 446	5	8	3	8	3	5	7	6	7	5	1	10	3	1	6	10	88
MT 41	9	5	10	4	10	8	10	5	5	10	10	7	2	9	10	7	121
MT 76	8	4	7	1	7	4	4	4	3	8	6	5	9	6	3	3	82
MT 66	2	9	2	3	6	6	3	3	1	2	9	1	5	4	4	5	65
MT 938	7	3	6	7	8	1	1	2	4	1	3	9	1	10	1	9	72
AC 650	6	10	9	5	5	9	5	7	6	4	2	8	6	5	8	2	97
AC 652	1	1	1	2	9	10	6	9	8	9	4	6	10	7	7	6	96
AC 728	4	6	5	10	2	7	2	1	2	6	5	3	7	3	2	4	69

GT - Genotypes
 PH - Plant height
 BD - Basal diameter
 NL - No. of leaves
 SLA - Single leaflet area
 GID - Girth increment %
 SC - Stomatal conductance
 TR - Transpiration rate
 CC - Chlorophyll content
 NS - No. of stomata
 PTT - Palisade tissue thickness
 SBP - Soft bast proportion
 SBL - Soft bast LVR %
 CR - Chlorophyll reduction %

Discussion

DISCUSSION

5.1 Genetic variability for cell membrane thermostability among wild *Hevea* germplasm

Generally in field condition, the plants are seldom exposed to single stress and they are often exposed to multiple stresses. In nature, temperature often interacts with water deficit and hence screening of a genotype either for water or high temperature stress alone will be of limited use for perennial tree crop like *Hevea*. Polyethylene-glycols (PEG) of high molecular weight have long been used to simulate drought stress in plants as a non-penetrating osmotic agent lowering the water potential in a way similar to soil drying (Bressan *et al.*, 1981; 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). Hence the laboratory screening method adopted in this study for osmotic and heat stress is a useful method of screening while dealing with a large number of germplasm materials. This is in agreement with Ashraf *et al.* (1996), who used cell membrane stability for screening and identified this as the most useful and efficient method for screening wheat germplasm for drought tolerance.

In the present study, the relative injury to cell membrane varied from 30-80 per cent with significant genotypic difference. The genotypes AC 446, AC 652 and MT 80 expressed very low injury to their cell membrane, indicating the ability of these genotypes to withstand heat and water stresses. Significant genotypic difference observed for cell membrane in *Hevea* is in accordance with earlier reports of Rajgopal *et al.* (1988) and Nair *et al.* (1995 and 1999). Significant varietal difference for this character was noticed in pepper leaves by Yao Yuangan *et al.* (1998). The significant genotypic difference for cell membrane stability indicates difference in their cellular sensitivity to desiccation stress. The genotypes coming under the extreme ranges indicate the availability of

broad genetic base in this germplasm material for this particular trait, which is highly useful for employing selection in further crop improvement programmes. Nagarajan and Bansal (1986) opined that the tissue conductivity method, which is relatively simple and uses only a few leaves, could be successfully applied to screen a segregating population.

The moderate genotypic coefficient of variability and a lower value of phenotypic coefficient of variability for this character, indicate that the influence of environment is less. Hence it is a trait that can be used in selection and breeding for crop improvement programme which is again supported by the high heritability value (87%) obtained for this character. Highly significant general combining ability (GCA) and specific combining ability (SCA) for cell membrane thermostability in common wheat reported by Xu Ruqiang *et al.* (1998) reflect the additive genetic value of this trait.

5.2 Genetic variability for drought related physiological parameters

The study was undertaken with an idea of assessing the genotypic performance under different levels of induced water stress. The effect of different intensities of water stress on the expression of certain physiologically related parameters was studied. This will lead to a better understanding of genotypic response to drought intensities as well as identification of drought related parameters, which ultimately reduces the task of further detailed studies in this field.

5.2.1 Leaf temperature

In this study, significant genotypic difference could be observed only in the stress (S-3) period, because in general the air temperature during the entire recording period was more or less constant (Appendix I). Even then, the different intensities of water stress had some effect on genotypes. Under the severe water

stress (S-3) period this difference was more clearly expressed and when this was compared with the corresponding non-stress period (NS-3) of irrigated plants, the genotypic difference, the different water levels as well as the interaction effect was significant. This indicates clearly the genetic variability among the wild genotypes.

The leaf temperature was increased in almost all the genotypes under S-3 level than non stress period. Increase in leaf temperature in the water stressed plants has been noticed in the field condition by Teeri (1980) which affect the metabolism via the kinetic properties of the enzymes and by heat injury (Berry and Bjorkman, 1980). Mid day stomatal closure has been observed in oil palm due to increase in leaf temperature (Rees, 1961). The highest leaf temperature of about 36°C observed in the wild genotype MT 76 indicates that this genotype does not reflect the visible light as effectively as others. Johnson *et al.* (1983) have correlated increased reflectance with the water balance maintenance by the reduction in leaf temperature. In coffee a negative relation between leaf temperature and stomatal conductance has been observed by Golberg *et al.* (1984). The impact of leaf temperature in the regulation of assimilation rate also has been reviewed by Farquhar and Sharkey (1982). Golhar and Dhopte (1996) have described the importance of leaf temperature as a parameter for identifying drought tolerant pigeon pea. Ranalli *et al.* (1997) identified the potential use of canopy temperature as a tool for drought screening of potato germplasm. Hence, variation observed in the present study among the wild germplasm for leaf temperature under different water stress levels indicates that the wide genetic variability is present in this material.

5.2.2 Components of water relation

5.2.2.1 Stomatal conductance

The highly significant genotypic difference obtained for stomatal conductance under each water level, clearly indicates the genetic variability present among the wild genotypes for this water relation component. The highest stomatal conductance recorded by the clone Tjir1 under normal condition, gives an indication of this clone being drought susceptible, whereas the wild genotype AC 652 is having more stomatal control indicated by the lowest stomatal conductance under non-stress. But this response was not constant when the stress intensity was increased. Under severe water stress (S-3), the genotype MT 41 exhibited a good stomatal control, whereby the stomatal conductance was reduced to the minimum, which was comparable to the standard clone RRII 105. The accepted drought tolerant clone RRIM 600 showed more conductance than this wild genotype. The higher stomatal conductance observed in the genotypes MT 66, MT 938 and AC 728, compared to the drought susceptible clone Tjir1 indicates the poor stomatal control of these genotypes under water stress conditions. All the genotypes responded very well to the irrigation given after the stress period. The higher stomatal conductance of RRIM 600 and AC 652 at post stress level indicates their most efficient way of water regulation by stomatal control.

A common response of crops to water stress is stomatal closure which reduces fluxes of both CO₂ and water vapour thus preventing leaf desiccation (Rajagopal and Sinha, 1979; Farquhar and Sharkey, 1982; Schulze, 1986). In wild *Hevea* genotypes, this impact is clearly seen during the stress period (S-3) among the genotypes AC 1044, MT 55, AC 446, MT 41, MT 76 and AC 728. All the standard clones RRIM 600, Tjir1 and RRII 105 showed the same trend but the reduction was more in RRII 105, which is in conformity with the earlier report of Rao *et al.* (1988). The reduction of stomatal conductance observed in most of the

genotypes, under water stress level is similar to the report of Zhang *et al.* (1997) where there was a reduction in stomatal conductance of ponderosa pine under water stress.

The maintenance of water balance in coconut by effective stomatal regulation has been reported by Milburn and Zimmermann (1977), Rajagopal *et al.* (1986), and Bai *et al.* (1988). Similar observation has been reported in other tree crops like cocoa (Balasimha *et al.*, 1988) and cashew (Balasimha, 1991). In *Hevea*, Cenlimans *et al.* (1983) and Chandrashekar *et al.* (1990) have reported the adverse effect of severe drought on stomatal conductance. The importance of considering the stomatal performance index for juvenile identification of drought tolerant *Hevea* clones has been identified by Chandrashekar (1997).

5.2.2.2 Transpiration rate

Water stress significantly reduced the transpiration rate. As in the case of stomatal conductance, the transpiration rate was also low in the wild genotype AC 652 and high in the clone Tjir1 which again support the previous result obtained for stomatal conductance. But when the stress intensity was increased to S-2 level, the minimum transpiration rate was recorded by the wild genotype MT 41 which was holding third position in the case of stomatal conductance. But for both parameters, the maximum was recorded by the wild genotype AC 728 under S-2 stress level. When the stress intensity was further increased to S-3 level, the minimum transpiration rate was recorded by the wild genotype AC1044 which was in the third position with respect to stomatal conductance under S-3 level. However, it is an indication that the wild genotypes MT 41 and AC 1044 are having good stomatal control during water stress condition, which is a useful indication for the selection programme. In addition to MT 41 and AC 1044 the other genotypes MT 55, AC 446 and AC 652 also exhibited a good stomatal control under water stress levels compared to the clone RRIM 600.

As the transpiration water loss from leaves is regulated by the stomata, transpiration showed a positive trend with that of stomatal conductance, i.e., with the increase in stomatal conductance, there was a concomitant increase in transpiration rate. A direct relationship between stomatal frequency and transpiration rate was shown in eight barley lines by Miskin *et al.* (1972). In coconut, comparatively lower transpiration rate was found in the drought tolerant types than in the susceptible types. Comparatively low transpiration rates observed in the wild genotypes MT 41 and AC 1044 under water stress levels indicate the possibility of them being drought tolerant genotypes.

The highest rate of transpiration recorded in the clone Tjir1 under non-stress condition is in conformity with the earlier report of Devakumar *et al.* (1988). This may be because of more heat absorption due to the low cuticle thickness in Tjir1 (Rao *et al.*, 1988). The lowest rate of transpiration recorded by the wild genotypes MT 41 and AC 1044 under various stress levels indicate their ability to maintain better plant water status. Close association between stomatal resistance and transpiration rate during stress period has been reported by Gummuluru *et al.* (1989) and hence lowest transpiration rate of MT 41 and AC 1044 may be due to the higher stomatal resistance or the lowest stomatal conductance obtained in the present study. The higher transpiration rate recorded in the wild genotypes AC 728 and MT 938 during stress period is due to the higher stomatal conductance or lower stomatal resistance. This observation leads to clonal variation in *Hevea* for stomatal behaviour.

5.2.2.3 Leaf water potential

Higher Ψ leaf has been used as an index for tolerance to drought in many crops like cotton (Ackerson, 1977), cocoa (Balasimha *et al.*, 1988) and coconut (Rajagopal *et al.*, 1988). Changes in Ψ leaf depend on both soil water supply and the evaporative demand of the atmosphere. Under field condition with

the progressive development of stress there is a reduction in Ψ leaf (Turner, 1974; Blum, 1974; Ehrlar *et al.*, 1978). In this study also, the Ψ leaf decreased with increasing water stress which was in conformity with the earlier report of Devakumar *et al.* (1988) for *Hevea* clones. The increase in stomatal resistance during stress period caused changes in Ψ leaf by changing transpiration rate (Passioura, 1982). So the low stomatal conductance and transpiration rate exhibited by the genotype MT 41 under stress period may be the reason for high Ψ leaf of this genotype under water stress (S-3) period.

The Ψ leaf of the drought tolerant clone RRIM 600 and the popular clone RRII 105 were significantly lower than this genotype at this water stress level. Hanson and Hitz (1982) inferred that Ψ leaf alone is not a generally satisfactory indicator of the plant water stress, and hence other than Ψ leaf, there are various other factors which determine the drought tolerance of RRIM 600 and RRII 105. Again, Devakumar *et al.* (1988) suggested that maintenance of higher Ψ leaf alone is not enough to explain completely the drought tolerance of *Hevea* in terms of yield. The genotypic difference noticed for ψ leaf in this study is in conformity with the earlier studies undertaken in various tree crops and this genetic variability is highly essential for employing selection for crop improvement programmes.

5.2.2.4 Soil water potential

With increasing intensity of water stress levels the Ψ soil recorded for *Hevea* genotypes decreased considerably which is in conformity with the earlier reports. This decrease in Ψ soil is reflected in the Ψ leaf of certain genotypes whereas in others, there was no direct relation between Ψ leaf and Ψ soil. This indicates that the wild genotypes react differently with different intensities of water stress and some genotypes extract more water from the soil to withstand the

stress conditions and thereby reducing the available Ψ soil. In *Hevea* one reason for low rubber yield during summer is soil moisture stress (Chua, 1970) and hence the differential response of wild *Hevea* genotypes to Ψ soil at stress condition is a positive indication of the scope of applying selection in this material for identifying suitable drought tolerant genotypes.

5.2.3 Chlorophyll fluorescence

Changes in chlorophyll fluorescence may well occur before any physical signs of deterioration are evident. Early indications of these changes allow the collection of valuable data on the onset of stress condition and the threshold of tolerance to increasing demands. Hence chlorophyll fluorescence measurements is a useful tool for rapid screening of large number of germplasm materials for stress tolerance.

Initial fluorescence (F_0) is known to be affected by any environmental stress that causes structural alterations at the photosystem II pigment level. Thermal damage of PS II is characterized by a drastic increase in F_0 . Here also, the F_0 under stress condition was drastically increased for all the genotypes whereas in the clones RR11 105 and RR1M 600 this drastic increase was comparatively lesser than other wild genotypes. In the drought susceptible clone Tjir1, the drastic increase of F_0 under stress level further confirms its drought susceptibility. The variations in the increase occurred for F_0 among the wild genotypes indicates their genetic variability for the thermal damage of PS II.

F_m , the maximal fluorescence, is decreased after exposure of leaves to high but not injurious temperatures. More severe stress results in an increase in F_0 and a decrease in F_m accompanied by inhibition of PS II activity. In the present study also, the F_m was decreased at water stress condition. In clone Tjir1 and in the wild genotypes MT 76 and MT 66 the inhibition of PS II activity might have occurred more as indicated by their high reduction of F_m . F_v , the variable

fluorescence ($F_m - F_0$) is usually lowered by environmental stress which causes thylakoid damage. The reduction of F_v under stress condition observed in this study also confirms this and the reduction was more in the clone Tjir1 and in the above mentioned genotypes, indicating their susceptibility towards drought stress.

In *Hevea brasiliensis* light induced inhibition of photosynthesis under drought and cold stress has been reported by Sathik *et al.* (1998) and drought induced photooxidative stress and inhibition in photosynthesis by Jacob *et al.* (1999). The ratio F_v/F_m has been shown to be proportional to the quantum yield of photochemistry (Butler and Kitajima, 1975) and shows a high degree of correlation with the quantum yield of net photosynthesis of intact leaves (Bjorkaman and Demmig, 1987). Hence, early detection of reduced photosynthetic capacity of the observed plants can be achieved non-destructively by the measurement of chlorophyll fluorescence. In this study, the wild genotypes AC 1044, MT 55 and AC 652 showed higher photosynthetic capacity as indicated by the less reduction of F_v/F_m under stress conditions. The F_v/F_m ratio of clones RRIM 600 and RRII 105 was also high whereas the same in Tjir1 was very low and was grouped with the wild genotypes MT 76, MT 66, MT 938 and AC 728 for their reduced photosynthetic capacity.

The ratio F_m/F_0 depends on leaf water potential and in drought conditions has been shown to drop to 1, i.e., no variable fluorescence will be produced after several days of drought. Here also, this ratio was above 3.0 for all the genotypes when they were under non stress condition but dropped to below or near to 1 as an effect of water stress. Much reduction was noticed in the wild genotypes MT 76, MT 66 and AC 728 and this was same for the clone Tjir1.

The drastic increase of F_0 and the reduction of F_m , F_v , F_v/F_m and F_m/F_0 observed under water stress condition in this study is in support of earlier reports. Chlorophyll fluorescence showed significant differences in cocoa at seasons and

between accession types (Balasimha, 1992; Balasimha and Daniel, 1994 and 1995). The F_v values in their study were lower during drier months as compared to other months. The F_0 was significantly higher in drought susceptible cocoa accessions. The results of this study suggest that relative chlorophyll fluorescence can be used for screening *Hevea* accessions for their drought tolerance.

5.3 Genetic variability for drought related morphological parameters

5.3.1 Field experiment

5.3.1.1 External appearance

In *Hevea* growth and yield are mainly affected under drought conditions and hence, clones with good growth and vigour even in the initial stages of growth are highly preferred for drought affected areas. The morphological characters like plant height, basal diameter, number of leaves, leaf area etc. give an indication of the general vigour of the genotype and hence these characters studied at the juvenile stage itself hold some importance. The significant genotypic difference observed in almost all the morphological characters studied among the wild genotypes indicated the wide genetic base of these material which is highly useful for crop improvement. The wild genotypes expressed the maximum genetic variation for almost all the characters recorded, whereas in the standard clones RRIM 600, RRII 105 and Tjir1 the genetic difference was nil except for number of leaves. This further indicates the scope of selection from the wild genotypes for crop improvement. Plant height and basal diameter are highly correlated and hence wild genotypes with more height will also express higher basal diameter. The wild genotype MT 41 possessed the maximum number of leaf flushes as well as the maximum number of leaves. Height and basal diameter of this genotype was also high, indicating the vigorous growth nature of this genotype.

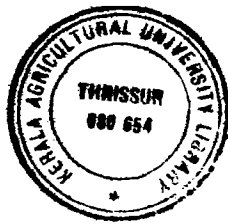
The variability observed in single leaflet area indicates the genetic variation present among these genotypes for the available transpirational area and all the control clones had lesser leaflet area. The reduction in leaf area helps the plants to adopt to periods of drought which might be due to the advantage in reducing transpiration. So the lowest leaf area recorded for the genotype MT 76 is an advantage for reducing the transpirational water loss. Variations in leaf expansion rate in cocoa accessions under water stress has been reported by Balasimha (1982). Conceicao *et al.* (1986) noticed a reduction in leaf number, flushes, shoot length and diameter in *Hevea* clones as a result of water stress. The interflush distance indicates the transportation distance and hence a low interflush distance is a positive sign where the translocation as well as partitioning of photosynthates will be more effective. In addition to that, they can provide mutual shading also. So the low interflush distance recorded in the genotypes MT 41, MT 76, MT 66 and AC 652 is more advantageous under drought stress conditions.

SLW did not differ significantly among genotypes, even though there was slight variation for this between these genotypes. Accessions with high SLW are preferred under drought conditions and hence the comparatively higher SLW noticed in the wild genotypes MT 55, MT 41, MT 76, MT 66, MT 938, AC 650 and AC 652 will be a valuable trait for germplasm material. Cocoa accessions with high SLW were found to be drought tolerant (Balasimha, 1987) and SLW is a good indication of leaf structure. In this study the majority of the genotypes with higher SLW appeared to be MT genotypes.

5.3.2 Glass house experiment

5.3.2.1 Effect of water stress on basal diameter

The reduction in basal diameter among the wild genotypes including control clones under water stress was as expected. The increase of basal diameter



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recorded under water stress in the genotype MT 41 is advantageous and indicates the merit of this genotype. The higher reduction percentage of basal diameter and greater growth depression of RRII 105 compared to RRIM 600 confirms the drought susceptibility of RRII 105 and drought tolerance of RRIM 600 with respect to growth. The lowest growth depression noticed in the genotype AC 652 highlights the worth of this genotype being suitable for a drought condition.

5.3.2.2 Effect of water stress on dry matter production

Leaf area and dry matter are the two plant characters that determine the total biological productivity, but partitioning of the total biological yield is the most important inherent character that determines the economical yield (Donald and Hamblin, 1976). Significant reduction in dry matter as a result of drought stress is reported in various crops like tea (Burgess and Carr, 1996), maize (Celiz *et al.*, 1995), field bean and field pea (Grzesiak *et al.*, 1997), coconut (Rajagopal *et al.*, 1989), eggplant (Byari and Al-Rabighi, 1996) and *Hevea* (Vijayakumar *et al.*, 1998).

Stress tolerance level can be understood by calculating DMSI. Low DMSI by the genotypes AC 652 and MT 41 indicates their ability to produce more dry matter even at water stress condition. The highest DMSI shown by the clone Tjir1 explains the reason of it being drought susceptible. The genetic variability present in the wild germplasm materials for DMSI again points out to the genetic potential of this materials which can be exploited well for drought resistance breeding.

5.4 Genetic variability for drought related biochemical parameters

The significant genotypic difference for the total ECW content present among the genotypes studied indicates the wide genetic variability present in these materials. ECW content in *Hevea* increases due to stress and helps the

plants to withstand drought (Vijayakumar *et al.*, 1998). The role of ECW in the maintenance of water balance has been reported in various crops such as cocoa (Balasimha *et al.*, 1995), rubber (Rao *et al.*, 1980) and coconut (Rajagopal *et al.*, 1988). Higher wax content helps in the adaptation of the plant to the drought conditions by reducing the stomatal conductance and cuticular transpiration. Among the wild genotypes, the highest ECW content expressed by MT 41 indicates the possibility of it being a drought tolerant one. This may be the reason for the highest leaf water potential of this genotype observed under water stress (S-3) level. In coconut Rajagopal *et al.* (1991) have observed a negative correlation of ECW content with transpiration rate. In addition to this, the presence of ECW on the leaf surface helps to reflect the excess solar radiation thereby maintaining the leaf temperature to a minimum level. Comparison of wax content was found to vary with species, seasons and also with intensity of light which will affect the cuticular transpiration as reported by Baker (1974) and Baker *et al.* (1979). From the significant genotypic difference observed for the wax production, it is clear that wide genetic variability is present among the wild *Hevea* germplasm material which can be utilised effectively in crop improvement programmes.

Chlorophyll is the major pigment which is affected during stress (Ludlow and Bjorkman, 1984). Reduction in chlorophyll content in unirrigated coconut palms during stress period was reported by Sivashankar *et al.* (1991) and in rainfed *Hevea* by Vijayakumar *et al.* (1998). The highest chlorophyll content observed for the wild genotype MT 41 again highlights the importance of this genotype for considering under drought stress situation. The genetic variation exhibited by the wild germplasm materials indicates the scope of identification of better genotypes performing under stress conditions.

The per cent reduction in total chlorophyll at a given high temperature, gives an idea about the stability of chlorophyll in the genotypes. The decrease in

chlorophyll content due to high temperature stress and water stress is due to loss of chloroplast membrane integrity under water deficit, which is correlated to enhanced activity of acid phosphatases localised on chlorophyll membrane. They also observed the perturbation in the structural organisation of chloroplast membrane causing a reduction in efficiency of the membrane dependent electron transport of photosynthesis. It has also been reported that majority of the chlorophyll loss in response to water loss occurred in the mesophyll cells and this loss was mainly due to reduction in the lamellar content of high harvesting chlorophyll a/b protein (Alberte *et al.*, 1977).

Chlorophyll reduction percentage is negatively correlated with yield and can be used to screen the genotypes for tolerance to heat. Hence low chlorophyll reduction percentage observed in wild genotypes AC 446, MT 938, AC 1044 and MT 41 is a good indication for better yield under high temperature. Though the total chlorophyll content in the control clone Tjir1 was more than in RRIM 600 and RRII 105, the chlorophyll reduction percentage was very high in Tjir1 whereas it was least affected in RRIM 600 and RRII 105. This may be one of the reasons for drought susceptibility of the clone Tjr1 and drought tolerance of RRIM 600 and RRII 105. Hence the wide genetic variations present in this aspect among the genotypes studied can be effectively utilised while dealing with large number of wild *Hevea* germplasm material for screening for drought tolerance. The superiority of the wild genotype MT 41 for the various biochemical aspects studied is noticeable where there was fairly high ECW and chlorophyll content and a comparatively less chlorophyll reduction percentage.

5.5 Genetic variability for drought related anatomical parameters

5.5.1 Leaf anatomical parameters

The photosynthetic machinery of the plant is situated in the green leaves and hence studying the leaf structural characters assumes special

importance. Stomatal pores, which are minute intercellular openings on the leaf surface, play an important role in the water balance system of the plant. Jones (1979) has reviewed the importance of stomatal studies in breeding for drought tolerance in crop plants. Since higher number of stomata per plant 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). Hence a comparatively less number of stomata present among the wild *Hevea* germplasm especially in the genotypes AC 446, MT 938, AC 650, MT 41 and AC 652 is advantageous. Since stomatal frequency is influenced by light, temperature and water stress, Ciha and Brun (1975) and Henzell *et al.* (1976) suggested that stomatal sensitivity to moisture stress should be taken as an indicator 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 Tjir1. Genetic variability has been demonstrated in various stomatal characteristics by Clarke and Smith (1986) which was true in the present study also.

The important leaf structural characters that take part directly or indirectly in the water regulation and gas 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. 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.

Midrib diameter is important for the translocation of photosynthates from sites of their production. Mesophyll tissue especially the palisade tissue are important for photosynthetic activity due to the presence of chloroplast in them. Because of this reason, the number of palisade cell per unit length of palisade tissue is important. Considering all these factors it is understood that the wide

genetic variability expressed by this germplasm materials is beneficial for assessing the genetic worth of each genotype while undertaking selection for particular situations. According to earlier reports the wild genotypes 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.

Accordingly the wild genotypes MT 76, AC 652, MT 938 and AC 1044 had higher leaf thickness. Mesophyll and palisade tissue thickness were also higher in these genotypes which indicate their higher photosynthetic capacity. Even though the leaf thickness and palisade and mesophyll tissue thickness were comparatively low in MT 41, palisade cell number per unit length of this genotype was higher whereas it was the lowest in the clone Tjir1. This may be one of the reasons for comparative drought tolerance of the wild genotypes MT 41 and the drought susceptibility of Tjir1.

5.5.2 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 George *et al.* (1980), Sethuraj and George (1976) and Sethuraj (1977). *Hevea* yield is mainly determined by the 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, Ho *et al.*, 1973, Ho, 1975 and Sethuraj, 1981) and in *Hevea* the rate of yield depression during drought period varies from clone to clone and the extend of variability is influenced by latex flow pattern (Sehuraj, 1976). Environmental factors such as soil moisture and atmospheric temperature cause turgor pressure variation in the laticifers which can influence the seasonal yield

variations mediated through total volume of latex as proposed by George *et al.* (1980).

The proportion of soft bast and proportion of LVR in the soft bast in *Hevea* clones give an indication of their response to drought and in earlier studies Premakumari *et al.* (1993) could clearly differentiate the drought susceptible and tolerant clone based on this aspect. Significantly high proportion of soft bast and LVR in soft bast were noticed in drought susceptible *Hevea* clones. Hence, the variation noticed among wild genotypes for these characters in the present study give an indication of their drought response. Too young age of the genotypes considered in the present study for bark anatomical characters may be the reason for the absence of significant genotypic variation. Usually in *Hevea*, bark anatomical characters are clearly expressed towards later stage than the juvenile stage. Even then the lowest soft bast proportion and the lowest LVR proportion in the soft bast noticed in the wild genotype MT 41 indicate the genetic potential of this genotype for drought tolerance.

5.6 Genetic parameters

A proper understanding of the genetic variation available in the population helps to identify the genetic potential of that population. Genetic parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in the broad sense (H^2) and genetic advance (GA) help in partitioning the genetic variability into heritable and non heritable components (Johansen, 1909).

In all the characters considered, the PCV was greater than GCV, indicating the influence of environment. However, the influence was comparatively less in the characters plant height, single leaflet area, leaf thickness, palisade tissue thickness, palisade cell number per unit length, bark

thickness and ECW content. PCV of stomatal conductance and transpiration rate was much lower than these characters which was clear from the high proportion of GCV value in relation to their PCV values. This indicates the involvement of genetic factors in the expression of these characters. This result is in conformity with the earlier results of Mercy *et al.* (1993) reported in a set of wild genotypes at 18 months age.

Broad sense heritability (H^2) is the proportion of total genetic variance to the total or phenotypic variance, which reflects the proportion of additive plus non-additive genetic variance and is useful in predicting improvement achieved by cloning selected trees (Hogarth, 1971). Heritability for all the characters considered was medium to high indicating the genetic influence on the expression of these characters. Leaf and bark structural characters, ECW as well as the physiological characters had very high H^2 , which can be effectively exploited in the selection programme for the crop improvement, as the heritable portion of this character is confirmed.

Heritability estimates along with genetic advance give a clear picture of the actual genetic improvement possible for that character. The traits with high heritability and genetic advance are controlled by additive gene action and are therefore amenable to genetic improvement by selection. Among the characters considered, the total number of LVR had highest genetic advance of 58.39 which indicates that *Hevea* yield can be improved by selecting genotypes with higher number of LVR. Bark thickness possessed the next highest GA and hence these two bark structural characters are less influenced by environment. All the morphological characters recorded medium genetic advance which is in conformity with earlier report by Mercy *et al.* (1993) which indicate the influence of environment for the expression of these characters and hence the improvement expected for these characters will be less. GA for leaf structural and physiological parameters considered was low to medium but the high heritability recorded in

these characters indicate the additive gene action, and hence considerable genetic gain can be expected for these characters when included in the selection programme. The very low GA expressed by the number of cells per unit length of palisade tissue indicate the non-additive gene action and hence no genetic gain is expected. Similar was the GA for ECW content which is highly dependent on environment.

5.7 Character associations

The nature and extent of relationship between characters in a population can be understood by studying the correlations among them. While applying selection for crop improvement, the characters associated with a particular character under selection also will get a chance to get selected and hence selection pressure can be exerted very efficiently in any one of these easily discernible characters. Many of the morphological characters had high positive correlations with each other making their selection more easy. Along with leaf thickness, palisade tissue thickness is also get selected which is useful for identifying a suitable genotype for drought condition. Similarly, when genotypes with more vigorous growth are selected, there is a chance for getting high yielders due to the positive association with LVR.

F_v/F_m ratio gives indication of photosynthetic efficiency and is showing high negative correlations with stomatal conductance and transpiration rate. Hence, when genotypes with low stomatal conductance and transpiration rate are selected for drought conditions, their photosynthetic efficiency will be more. The negative genotypic and phenotypic correlations of leaf temperature with stomatal conductance may be due to the transpirational cooling occurring on the leaf surfaces.

High positive genotypic correlation of leaf water potential with epicuticular wax content may be due to the reduction in transpiration rate when

ECW is present on the leaf surface. These character associations help a plant breeder to conduct sensible selection among genotypes based on the need.

5.8 D² analysis

Understanding of genetic divergence of a population is a useful prerequisite where the breeder can identify the most distant group as parents for hybridization. The wild *Hevea* germplasm material is a good resource of genetic variability and hence more groupings are expected here which was obtained in the present study. The grouping of 10 wild genotypes in to 6 clusters in both the clustering may be due to the large number of characters considered. When the grouping was done based on morphological and structural characters some of the wild genotypes were grouped along with the control clones RRIM 600 and RRII 105, indicating the genetic similarity of these genotypes with the standard clones for these characters. But when the grouping was based on physiological and biochemical characters, the standard clones RRIM 600 and RRII 105 grouped distinctly into a separate cluster. This indicates the dissimilarity between wild genotypes and the standard clones for characters associated with physiological and biochemical aspects of the plant. In both the clustering the clone Tjir1 was grouped along with the wild genotype, which clearly indicates the different drought response of this clone in comparison to the control clones RRIM 600 and RRII 105.

5.9 Identification of superior genotypes based on rank sums

When the genotypes differ from each other for different drought related parameters, a ranking based on the parametric relationship with drought tolerance gives a better understanding of the worth of individual genotypes. Hence, some of the highly related parameters with drought tolerance were selected and genotypes were ranked based on their performance under each selected character. Such a ranking was adopted in cocoa accessions, while going for screening drought

tolerance among these accessions (Balasimha *et al.*, 1988). The highest rank sum (121) obtained for MT 41 indicates the relative superiority of this genotype for drought tolerance. The genotypes next to MT 41 are MT 55 and AC 650. The three control clones (RRII 105, RRIM 600, Tjir1) selected for the study and the superior genotypes MT 41 and MT 55 identified from the present study are shown in Plates 22 and 23 respectively. The lowest rank sum obtained for MT 66 (65) indicates its poor performance for this aspect and hence when we go for further selection or further studies, it can be concentrated with genotypes MT 41, MT 55 and AC 650 where the rank sums were higher.

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Plate 22. Control clones Tjir1, RR11 105 and RRIM 600 selected for the study



Plate 23. Superior genotypes MT 41 and MT 55 identified from the present study



Summary

SUMMARY

This study was conducted with 99 wild *Hevea* germplasm lines conserved in the Rubber Research Institute of India, Kottayam for a period of three years from 1998-2000. The main objective of the study was to assess the genetic variability present in this germplasm in relation to drought tolerance. Various physiological, morphological, biochemical and anatomical characters were examined under non stress as well as induced water stress conditions. Based on the results, superior genotypes among the selected accessions and reliable parameters for drought tolerance screening were identified. The results are summarised below.

1. Cell membrane thermostability among the selected 99 wild *Hevea* genotypes showed wide variation and the genotypic difference was significant. The relative injury to cell membrane varied from 30-80 per cent with a mean of 53 per cent. Genotypes AC 446, AC 652 and MT 80 had very low injury to their cell membranes. This trait had a moderate GCV of 22 per cent and a very high heritability of 87 per cent. The genetic advance obtained was moderate (22%).
2. On the basis of relative injury to cell membrane, the wild genotypes AC 652, AC 446, MT 55, MT 66, MT 41, MT 76, MT 938, AC 1044, AC 650 and AC 728 were selected for further detailed studies along with control clones RRII 105, RRIM 600 and Tjir1.
3. The genotypes recorded a higher leaf temperature under water stress condition and analysis of data revealed significant difference among genotypes as well as between stress and non-stress conditions.
4. Among the components of water relation, the stomatal conductance was reduced under stress condition and significant genotypic difference was noticed for this. When data were analysed for non stress vs stress

levels, genotypic difference, difference between non stress and stress and the interaction effect between genotype x non stress vs stress were all significant.

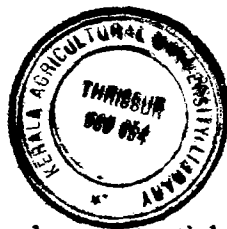
5. Transpiration rate also expressed significant genotypic difference under each stress level, and the rate of transpiration was reduced, when the genotypes were grown under stress condition. Here also, the genotypic difference, NS vs S and interaction between genotype x NS vs S were all significant.
6. Leaf water potential of the genotypes ranged from -3.15 to -2.74 MPa. With increasing intensities of water stress the leaf water potential went on decreasing.
7. Soil water potential under each genotype ranged from -1.76 to -0.314 MPa and here also Ψ soil decreased under water stress. Genotypic difference, NS vs S period effect, as well as the interaction between genotype x NS vs S were all significant when stress and non stress conditions were compared.
8. The components of chlorophyll fluorescence namely initial fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v) and the ratios F_v/F_m and F_m/F_v were studied under water stress and non stress conditions. The F_0 showed a drastic increase under water stress and all the other components showed a reduction under water stress. The genotypic difference was significant under both stress and non stress conditions.
9. The morphological characters studied include plant height, basal diameter, number of flushes, total number of leaves, interflush distance, single leaflet area and specific leaf weight. All these characters exhibited significant genotypic difference except for SLW and interflush distance.

10. Under water stress, the basal diameter of most of the genotypes were reduced and the rate of change was significant.
11. When the genotypes were studied for their drymatter stress tolerance index (DMSI), there was significant genotypic difference and the highest DMSI was noticed in AC 652.
12. Epicuticular wax content of the genotypes varied significantly.
13. Chlorophyll content and chlorophyll reduction percentage as a result of heat treatment also varied significantly among the genotypes.
14. The stomatal density among the wild genotypes was 276.99-481.48 and the genotypic difference was significant.
15. Leaf thickness ranged from 104.23-129.83 μm and the genotypic difference was significant.
16. Midrib diameter range was 370.67-438.9 μm and differed significantly among the genotypes.
17. Palisade tissue thickness was in the range of 42.42-64.65 μm and here also the genotypic difference was significant.
18. Mesophyll tissue thickness differed significantly among the genotypes and the range was 84.95-114.52 μm .
19. Palisade cell number per unit length recorded varied from 24.29-35.24 and the genotypic difference was significant.
20. Bark thickness range among the wild genotypes studied varied from 1.277-1.623 mm, and there was no significant genotypic difference. The reason for this could probably be attributed to the young age of the plants.
21. Soft bast thickness ranged from 0.57-0.77 mm and the proportion of soft bast was 39-57 per cent.
22. Total LVR observed was in the range of 1.33-3.33 and the proportion of LVR in the soft bast ranged from 63-79 per cent.

23. Among the characters studied, stomatal conductance and transpiration rate exhibited fairly good GCV.
24. Most of the morphological characters had high PCV. All the leaf and bark structural characters had low to medium PCV indicating less influence of environment for the expression of these characters.
25. Broad sense heritability (H^2) indicated medium to high values for all the characters considered.
26. Genetic advance (GA) as percentage of mean was low to medium for almost all the characters studied, except for LVR, where the GA was high.
27. Character association studied helped to understand the relationship of various characters with each other.
28. Genetic divergence was worked out separately for (a) morphological and structural characters, (b) for physiological and biochemical characters. In both, the genotypes were grouped into 6 clusters each indicating the wide genetic distance among the genotypes.
29. The ranking of each genotype based on parametric relationships with drought tolerance helped to identify the actual worth of each genotype, and based on rank sum obtained, the best genotype identified was MT 41 followed by MT 55 and AC 650.

Conclusion

The ability of a plant to withstand water deficit is associated with numerous plant traits that contribute to drought tolerance. An understanding of those characters which are more directly related to drought tolerance helps in easy identification of genetic materials which can be used for extensive study in this area. The breeder has to be mindful of the best combination of traits and the appropriate response strategy for the target environment. Genetic resource like wild *Hevea* germplasm material where there is wide genetic variation for each



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individual character provide the breeder a potential genetic stock to work with, in the efforts for developing drought tolerant *Hevea* clone.

In the present study a screening for drought tolerance among wild *Hevea* germplasm was carried out using various physiological, morphological, biochemical and anatomical indices. The selected accessions expressed significant genotypic differences for most of the parameters studied and their response under various water stress levels was significant. This shows the wide genetic base of these material, which provide ample scope to identify superior genotypes. These can be used as potential parents in future breeding programmes for developing varieties suitable for drought stress conditions.

The study reveals that a genotype associated with physiological drought tolerance trait need not have anatomical, biochemical or morphological traits for the same character. Girth increment during stress period and dry matter stress tolerance index were found to be useful for identifying a genotype showing drought tolerance. Similarly rate of stomatal conductance during water stress period, F_v/F_m ratio of chlorophyll fluorescence parameter, epicuticular wax content on the leaf surface and chlorophyll content, were some of the reliable physiological and biochemical indices for identifying a drought tolerant genotype. Among the leaf structural parameters studied, palisade tissue thickness and palisade cell number per unit length gave an indication towards drought tolerance.

Based on the above parameters, the wild genotypes MT 41, MT 55, and AC 650 were identified as accessions possessing characters related to drought tolerance. In this way, the identified objectives of the present study could be fulfilled more or less completely, though drought resistance is a complex factor involving a number of physiological, morphological, biochemical and anatomical characteristics with unknown inheritance. The nature and importance of these characters will vary depending on the pattern, degree and timing of the stress. Hence, for evaluating a large number of genotypes for drought resistance, the measuring technique used must be simple and fast.

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* Originals not seen

Appendix

APPENDIX-I
 Monthly weather data during the peak summer periods of 2000

Month	Air temperature (°C)		Mean vapour pressure (mm)	Mean relative humidity (%)	Total rainfall (mm)	Number of rainy days	Mean sunshine hours
	Maximum	Minimum					
March	33.7	23.20	22.20	77.0	118.22	4	8.3
April	33.1	24.90	23.95	80.5	97.30	11	6.8
May	33.6	24.60	23.05	77.0	57.90	7	8.3

**GENOTYPIC EVALUATION AND SCREENING FOR
DROUGHT TOLERANCE IN WILD *Hevea* GERMPLASM**

By

M.A. MERCY

ABSTRACT OF THE THESIS
Submitted in partial fulfilment of the
requirement for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

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ABSTRACT

Rubber tree (*Hevea brasiliensis* Muell. Arg.) is the only commercial source of natural rubber and the species is well suited to the equatorial region with plenty of well distributed rainfall and minimum fluctuations in temperature. With increasing global demand for natural rubber, attempts have been made to extend the cultivation of this tree to agroclimatically marginal lands such as drought and cold prone areas. But the very narrow genetic base of the cultivated *Hevea* resulted from the development of the species from a limited number of seeds introduced by Sir Henry Wickham, limits the scope of extending the clone to such marginal areas. A broad genetic base is a prerequisite for developing new varieties tolerant to various stress conditions. Plant genetic resources provide the requisite genetic variability and are the most important and vulnerable basic materials to meet the current and future needs of plant breeding programmes.

The wild germplasm accessions collected by an expedition of International Rubber Research and Development Board (IRRDB) during 1981, into the primary centre of origin of the crop, the Amazon forests provide a rich source of natural variability in this tree species. Introduction of genes from such wild progenitors is an ideal method of broadening the genetic base of cultivated *Hevea* species.

To make more efficient use of these germplasm materials, it is necessary to identify morphophysiological traits and structural and biochemical traits associated with tolerance to different abiotic stresses in these materials. Such a detailed study for traits associated with drought tolerance has not been made so far using wild *Hevea* germplasm materials. Assessing the genetic variability for such traits in these germplasm materials will help further to identify superior genotypes which can be used as potential parents in future breeding programmes for developing varieties suitable for drought stress conditions. Hence

the proposed research work entitled “Genotypic evaluation and screening for drought tolerance in wild *Hevea* germplasm” was carried out with the objectives of assessing the genetic variability present among the wild *Hevea* germplasm materials for characters associated with drought tolerance and screening of wild *Hevea* germplasm to identify genotypes for future breeding programmes of developing a drought tolerant clone.

The entire study was conducted with 99 wild *Hevea* germplasm lines conserved in the Rubber Research Institute of India, Kottayam for a period of three years from 1998-2000. Three separate experiments were carried out. In Experiment I a preliminary screening of 99 wild *Hevea* germplasm accessions (representative of Acre and Mato Grosso states of Brazil) for drought tolerance based on cell membrane stability was conducted using RRII 105 as control clone. The relative injury to cell membrane varied from 30 to 80 per cent with a mean of 53 per cent and the heritability (H^2) for this character was very high (87%). Based on relative injury to cell membrane, the entire accessions were classified and 10 accessions (AC 1044, MT 55, AC 446, MT 41, MT 76, MT 66, MT 938, AC 650, AC 652, AC 728) representative of all classes were selected for detailed study under Experiment II along with the control clones RRII 105 (a popular high yielding clone), RRIM 600 (a drought tolerant clone) and Tjir1 (a drought susceptible clone).

Experiment II was conducted under field conditions to assess the genetic variability among the 10 selected accessions for drought tolerance using various indices. The polybag plants were grown under induced water stress for 15 days, 30 days and 45 days. Drought related physiological parameters viz., leaf temperature, stomatal conductance, transpiration rate, afternoon leaf and soil water potentials and chlorophyll fluorescence parameters were studied along with the plants under non-stress. Under water stress condition, leaf temperature was increased and stomatal conductance, transpiration rate and afternoon leaf and soil

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water potentials were decreased. Components of chlorophyll fluorescence viz., initial fluorescence (F_0) showed a substantial increase under water stress and all other components - maximal fluorescence (F_m), variable fluorescence (F_v), F_v/F_m and F_m/F_0 showed a remarkable decrease. The selected accessions exhibited significant genotypic difference for all the physiological parameters studied and the effect of water stress and non stress on genotypes as well as the interaction effect between genotype and stress levels were also significant for most of the characters. Significance of genotypic difference was worked out statistically following Completely Randomised Design (CRD) and Factorial CRD.

Morphological, biochemical and anatomical (leaf and bark) parameters related to drought tolerance were recorded from the polybag plants under non stress conditions, in order to assess the genotypic difference for these parameters. Morphological characters studied include plant height, basal diameter, number of flushes, total number of leaves, inter flush distance, single leaflet area and specific leaf weight. All these characters exhibited significant genotypic difference except for specific leaf weight and inter flush distance.

Epicuticular wax content, chlorophyll content and chlorophyll reduction percentage were studied under biochemical parameters. Here also there was significant genotypic difference for all these characters.

Leaf anatomical characters studied include stomatal density on the lower surface, leaf thickness, midrib diameter, mesophyll tissue thickness, palisade tissue thickness and palisade cell number per unit length of palisade tissue. All these exhibited significant genotypic difference.

Total bark thickness, proportion of soft bast, total number of latex vessel rows (LVR) and proportion of LVR in the soft bast were the bark structural characters studied. There was no significant genotypic difference for these bark

structural characters which might probably due to the juvenile stage of the plants selected.

A glass house study was conducted as Experiment III in order to avoid the influence of untimely rains occurred during the recording period. Observations on growth of basal diameter and dry matter production under stressed and non stressed conditions were studied. Under water stress, the basal diameter of most of the genotypes was reduced and the rate of change was significant. On the basis of dry matter produced, dry matter stress tolerance index (DMSI) was worked out which showed significant genotypic difference. The highest DMSI was noticed in the wild accession AC 652.

Genetic parameters viz., phenotypic and genotypic coefficients of variability (PCV and GCV), broad sense heritability (H^2) and genetic advance (GA) as percentage of mean were worked out for the selected characters. As expected most of the morphological characters had high PCV and the other parameters had low to medium PCV indicating less influence of environment for the expression of these characters. Among the characters studied, physiological parameters viz., stomatal conductance and transpiration rate exhibited fairly good GCV. Broad sense heritability (H^2) indicated medium to high values for all the characters considered. Genetic advance (GA) was low to medium for almost all the characters studied, except for LVR where the GA was high (58%).

Character association studied helped to understand the relationship of various characters with each other. Genetic divergence existing in the population of wild genotypes was assessed in terms of "generalised group distance" using Mahalanobis D^2 analysis. Two separate analyses were done (a) for morphological and structural characters and (b) for physiological and biochemical characters. In both, the genotypes were grouped into 6 clusters each indicating the wide genetic distance among the genotypes.

A ranking of these genotypes was done for a better understanding of the worth of individual genotype, as they differed from each other for different drought related parameters. Some of the highly related parameters with drought tolerance were selected and based on their relationship with drought tolerance, genotypes were ranked for each selected character. Comparing the rank sum obtained for each genotype, the superiority of the accessions was assessed. Based on this, the best genotype identified was MT 41 followed by MT 55 and AC 650.

From this study it is concluded that drought resistance mechanism is a complex factor involving a number of physiological, morphological, biochemical and anatomical parameters with unknown inheritance. An understanding of these characters, which are more directly related to drought tolerance helps in easy identification of genetic materials which can be used for extensive study in this area. From the present study it was found that, the parameters such as stomatal conductance under water stress, F_v/F_m ratio of chlorophyll fluorescence, growth of basal diameter under water stress, chlorophyll and epicuticular wax contents, thickness of palisade tissue and palisade cell number per unit length of palisade tissue are some of the reliable parameters for identifying genotypes having drought tolerance. Extensive studies using these parameters are further required in this area, to identify a simple and fast screening technique for the large number of *Hevea* germplasm.