IDENTIFICATION OF POTENTIAL DROUGHT TOLERANT *HEVEA* GERMPLASM ACCESSIONS USING PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

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Hevea brasiliensis is the most important commercial source of natural rubber. Its cultivation is being extended to drought prone non traditional areas to meet the increasing global demand. The wild germplasm accessions of *Hevea* collected from its primary centre of origin, the Amazon forests, are valuable source of genes conferring tolerance to various biotic and abiotic stresses. Identifying suitable germplasm accessions with stress tolerance and yield sustainability would be important to enhance crop productivity. In the present study, a set of 18 *Hevea* germplasm accessions (14 relatively drought tolerant and 4 relatively drought susceptible), short listed based on previous observations were further evaluated for their drought tolerance potential using physiological and biochemical parameters such as photosynthetic rate, chlorophyll fluorescence, leaf wax content and pigments. The data revealed wide genetic variability among the accessions as indicated by a wide range obtained for these characters in the ranking. The accessions were ranked for their pooled performance using the rank sum method and the results indicated that accessions RO 3261, AC 612 and RO 3157 are the top three drought tolerant accessions among the lines studied.

Keywords: Carotenoids, Chlorophyll, Drought, Epicuticular wax, Fluorescence, Germplasm, Photosynthesis

INTRODUCTION

Hevea brasiliensis, the commercial source of natural rubber (NR), is widely cultivated in south-east Asian countries like Malaysia, Thailand, Indonesia, India, China, Sri Lanka and Vietnam. In India, the traditional rubber growing regions include Kerala State and Kanyakumari District of Tamil Nadu where the climatic conditions are more favourable for NR cultivation. The non-traditional regions in India include drought prone areas such as North Konkan regions, parts of Karnataka, Odisha, Madhya Pradesh and low temperature prevailing areas in the north-eastern states. To cope with the increasing global demand for NR, its cultivation needs to be extended to these non-traditional regions which warrant identification of suitable clones that can perform well in such regions.

Plant responses to water deficit stress are complex phenomena encompassing many

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aspects including stress sensing and signaling; changes in growth and biomass allocation patterns; decreased stomatal conductance and CO_2 assimilation; osmoregulation and detoxification processes (Chaves *et al.*, 2003). Plants respond to drought primarily by closing the stomata resulting in reduction of water loss and restriction of CO_2 diffusion leading to substantial reduction in assimilation (Lawlor and Tezara, 2009). Chlorophyll fluorescence measurements represent a sensitive and reliable method to monitor the changes caused by drought stress in crop plants. Silva *et al.* (2007) has shown that parameters like chlorophyll fluorescence, chlorophyll index and leaf temperature can be used as potential tools to rapid and non-destructive screening for drought tolerance in sugarcane.

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both the chlorophyll *a* and *b* are reported to be photo oxidized under soil moisture deficit condition (Farooq *et al.*, 2009). Under water stress condition, photosynthetic pigments and electron transport components were changed (Anjum *et al.*, 2003), photosynthetic apparatus damaged (Fu and Huang, 2001) and activities of enzymes in Calvin cycle also diminished, which leads to reduction in crop yield. Amount of epicuticular wax on the leaf surface is reported to be an important parameter associated with drought and heat tolerance (Rajagopal *et al.*, 1990).

Wild relatives of cultivated species are potential source of drought tolerance in several crops (Shimshi *et al.*, 1982). The wild accessions of *Hevea* (about 4500) collected from its primary centre of origin, the Amazon forests, is an excellent repository of various useful traits including drought tolerance. Many *Hevea* germplasm lines have been already identified with moderate to good drought tolerance potential (Nair *et al.*, 2005; Mercy *et al.*, 2009; 2010). In the present study, drought tolerance potential of 18 *Hevea* germplasm accessions was evaluated using specific physiological and biochemical parameters and the results are discussed.

MATERIALS AND METHODS

Six month old polybag plants of 18 (14 relatively drought tolerant and 4 susceptible) germplasm accessions, short listed based on extent of leaf yellowing (Nair *et al.*, 2011) were selected for this study along with relatively drought tolerant (RRIM 600 and RRII 430) and susceptible (RRII 105 and RRII 414) check clones (Sumesh *et al.*, 2011). The plants (n=5) were subjected to water deficit stress by withholding irrigation for seven days during the summer season of 2013. The net photosynthetic rate (P_N) and chlorophyll fluorescence were measured using portable photosynthesis system (LI-6400, LI-COR, USA) at a fixed CO_2 concentration of 400 ppm and light intensity of 500 μmol $m^{-2}s^{-1}$ provided using the leaf chamber fluorometer (LCF-40, Li-COR, USA) attached to the photosynthesis system.

Leaf samples were collected on the seventh day of drought imposition for biochemical analyses. Physiologically mature leaves were collected from the selected plants for estimation of pigments and epicuticular wax contents. The leaf epicuticular wax content was determined according to the method described by Ebercon *et al.* (1977). Total cholophyll was estimated by the method of Arnon (1949) and the carotenoids were estimated according to Lichtenthaler (1987).

Significance of genotypic difference for different parameters was worked out statistically by ANOVA. The accessions were evaluated for their drought tolerance potential by ranking them using rank sum method.

RESULTS AND DISCUSSION

Specific parameters associated with drought tolerance traits were studied in germplasm accessions after exposing them to water deficit stress for one week period. Withholding water for one week period was sufficient to initiate drought response in polybag plants as ascertained from our previous studies (Sumesh *et al.*, 2011). These accessions were selected from a previous

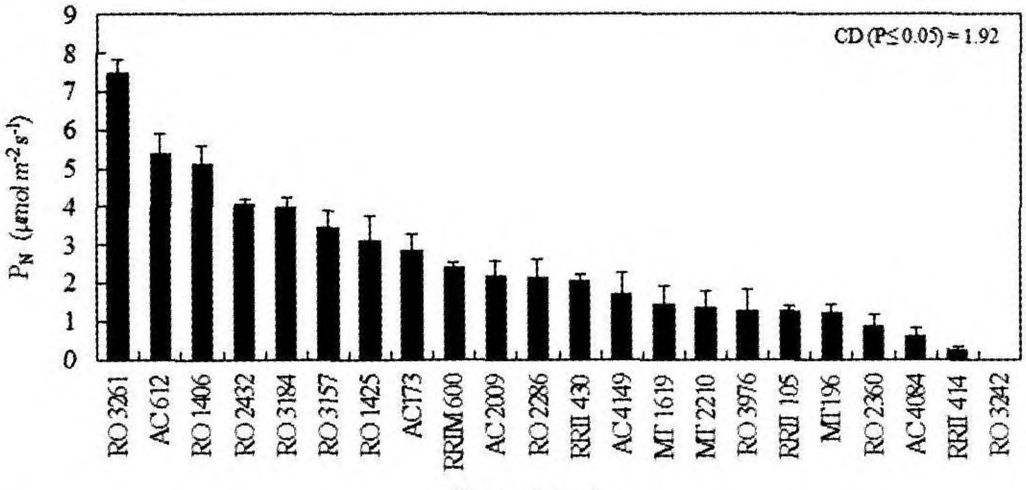

Fig. 1. Photosynthetic rate (P_N) in different *Hevea* genotypes after drought imposition for seven days

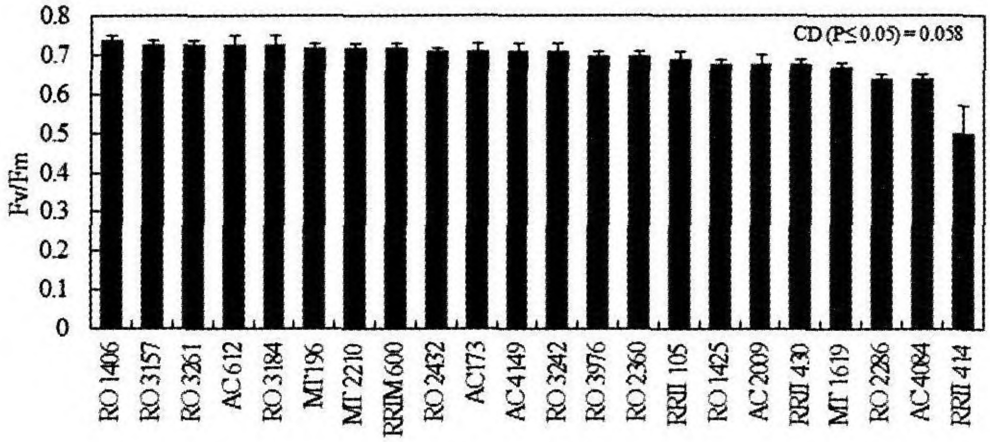

Fig. 2. Maximum potential quantum yield of PS II (Fv/Fm) in different *Hevea* genotypes after drought imposition for seven days

study on screening of germplasm lines based on extent of leaf yellowing (Nair *et al.*, 2011). The wild accessions used in this study were found to show varied response to the different drought related parameters *viz.* photosynthetic rate, maximum quantum yield, leaf wax content and pigments.

The data on photosynthetic rate (P_N) is given in Figure 1. The photosynthetic rate of the genotypes under drought conditions varied significantly which ranged from zero to 7.51 µmol $m^{-2} s^{-1}$. The accession RO 3261 recorded the highest and RO 3242 the lowest P_N. Eight accessions showed higher P_N than the drought tolerant check clone RRIM 600. The data on maximum quantum yield of PSII (Fv/Fm) is given in Figure 2. The accessions showed significant genotypic difference for Fv/Fm. The accession RO 1406 recorded the highest and AC 4084 the lowest values. Two accessions showed higher Fv/Fm than RRIM 600.

Significant genotypic differences for various drought related characters were noticed in *Hevea* (Nazeer *et al.*, 1992). Measurements of gas exchange and chlorophyll fluorescence parameters in plants during water and high temperature stresses showed that water deficit (17-20%) caused a significant decrease in the rate of CO_2 uptake and O_2 evolution (Yordanov *et al.*, 1998). During stress period, absorption of light is likely to exceed that required for photosynthetic assimilation and can lead to photo inhibition of the photosynthetic apparatus (Methy *et al.*, 1996; Jacob *et al.*, 1999). Drought tolerance capacity has been found to vary among different *Hevea* clones (Annamalainathan *et al.*, 2010). Photosynthetic gas exchange parameters in *Hevea* showed a declining trend under water deficit stress conditions (Sangsing *et al.*, 2004; Alam *et al.*, 2006; Sumesh *et al.*, 2011). The maximum potential quantum yield is

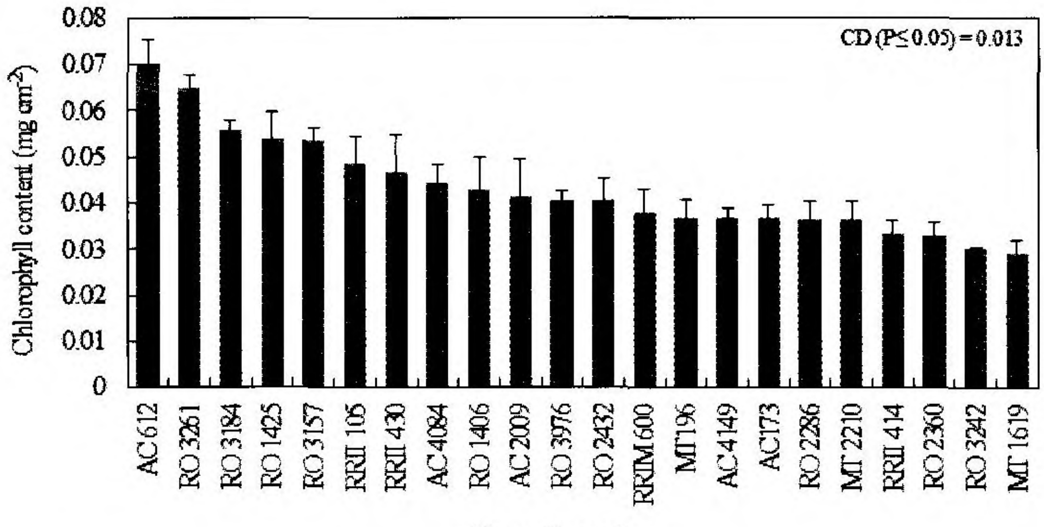

Fig. 3. Total chlorophyll in different *Hevea* genotypes after drought imposition for seven days

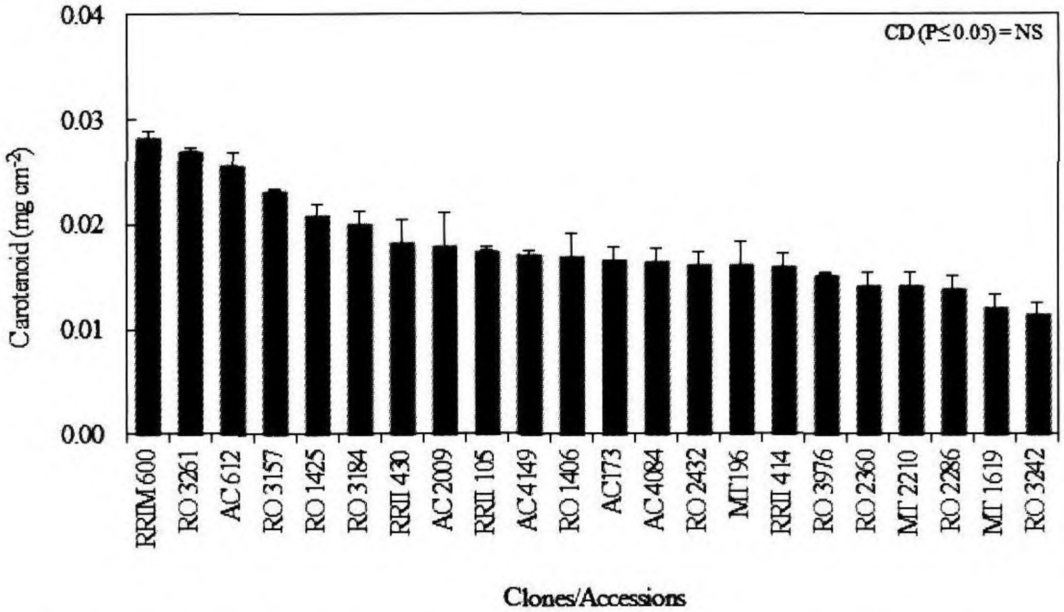

Fig. 4. Carotenoid content in different *Hevea* genotypes after drought imposition for seven days

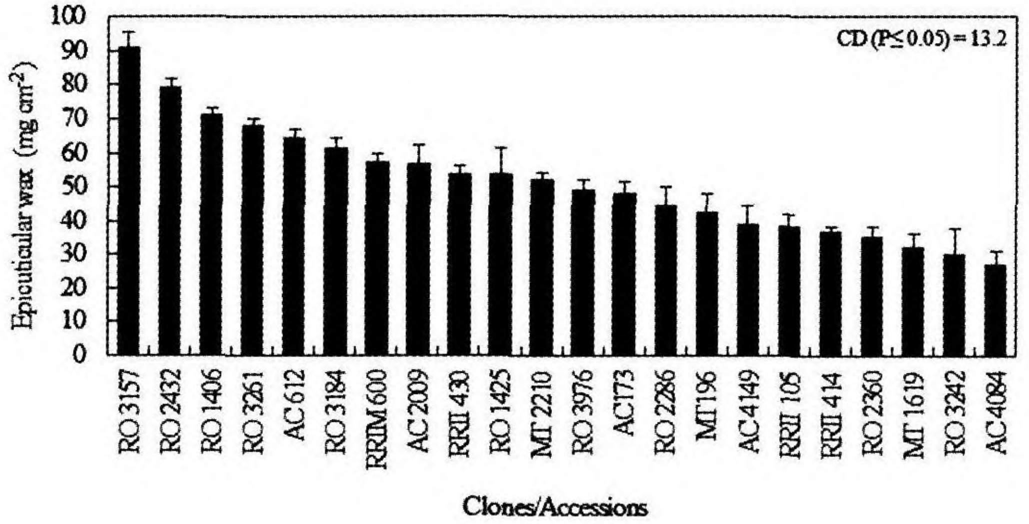

Fig. 5. Epicuticular wax content in different *Hevea* genotypes after drought imposition for seven days

Table 1. **Ranking of selected genotypes of *H. brasiliensis* based on parameters related to drought tolerance**

Clone/ Accession	Photo- synthesis	Fv/Fm	Wax	Total chlorophyll	Carotenoids	Total	Rank
RO 3261	21	20	18	20	20	99	1
AC 612	20	20	17	21	19	97	2
RO 3157	16	20	21	17	18	92	3
RO 3184	17	20	16	19	16	88	4
RO 1406	19	21	19	13	11	83	5
RRIM 600	*13*	*19*	*15*	*9*	*21*	*77*	*6*
RO 1425	15	15	12	18	17	77	6
RO 2432	18	18	20	10	8	74	7
RRII 430	*10*	*15*	*13*	*15*	*15*	*68*	*8*
AC 2009	12	15	14	12	14	67	9
AC 173	14	18	9	6	10	57	10
RRII 105	*6*	*16*	*5*	*16*	*13*	*56*	*11*
AC 4149	9	18	6	7	12	54	12
RO 3976	6	17	10	11	5	49	13
MT 196	4	19	7	8	7	45	14
MT 2210	7	19	11	4	3	44	15
RO 2286	11	13	8	5	2	39	16
AC 4084	2	13	0	14	9	38	17
RRII 414	1	12	4	13	6	36	18
RO 2360	3	17	3	1	4	28	19
MT 1619	8	14	2	0	1	25	20
RO 3242	0	18	1	1	0	20	21

an important and reliable measure of the structural and functional integrity of PSII under a given environmental condition. Nair *et al.* (2005) used this as a reliable parameter for drought tolerance potential in wild *Hevea* germplasm accessions.

The results on pigment analyses (total chlorophyll and carotenoids) are given in Figures 3 and 4, respectively. The accessions showed significant genotypic difference for total chlorophyll content in leaves. The accession AC 612 recorded the highest where as MT 1619 recorded the lowest chlorophyll content. The check clone RRIM 600 recorded the highest carotenoid content. Among the accessions, RO 3261 and RO 3242 recorded the highest and the lowest carotenoid contents, respectively. Cartenoids are essential components in the photosynthetic apparatus in plants where they protect against photo oxidative damage and contribute to the light harvesting in photosynthesis (Goodwin, 1980).

The variability present among the accessions for the epicuticular wax content (ECW) is shown in Figure 5. The total wax content of the genotypes varied significantly which ranged from 91.13 µg cm^{-2} to 27.26 µg cm^{-2}. The highest ECW content was present in the accession RO 3157 (91.13 µg cm^{-2}) followed by RO 2432 (79.59 µg cm^{-2}) where as the lowest was in the accession AC 4084 (27.26 µg cm^{-2}). Six accessions showed higher wax content than RRIM 600. The role of ECW in the maintenance of water balance has been reported in various crops (Rajagopal *et al.*, 1990; Zhang *et al.*, 2005) and in wild accessions of *Hevea* (Nair *et al.*, 2005). Higher wax content helps in the adaptation of the plant to drought conditions by reducing the stomatal conductance and transpiration rate (Rao *et al.*, 1988). Jefferson *et al.* (1988) observed increased wax production in drought stressed alfafa plants and identified it as a potential drought resistance selection criterion.

Ranking of accessions

Tolerance to drought is a highly polygenic character involving various morphological, anatomical and physiological traits. The accessions were ranked based on five different parameters *viz.* photosynthetic rate, maximum quantum yield of PSII, total chlorophyll, carotenoid and epicuticular wax content. The rank sum for the accessions and their ranks are shown in Table 1. Five accessions (RO 3261, AC 612, RO 3157, RO 3184 and RO 1406) scored higher ranks than the drought tolerant check clone RRIM 600. RO 3242 and MT 1619 were the accessions which came in the bottom ranks (more susceptible). The top three accessions RO 3261, AC 612 and RO 3157 were selected as potential accessions with relatively better drought tolerance capacity. These accessions could be used in breeding programmes after further detailed evaluation of their level of drought tolerance.

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