DROUGHT INDUCED CHANGES IN LEAF PIGMENTS AND OSMOLYTE CONTENTS IN *Hevea* GERMPLASM ACCESSIONS

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ABSTRACT

The cultivation of *Hevea brasiliensis*, the major commercial source of natural rubber (NR), is being extended to drought prone non-traditional areas which require identification of suitable varieties that can perform well in such regions. The screening of *Hevea* germplasm accessions may be worth for identifying drought tolerant traits. In the present study, six relatively drought tolerant and four susceptible germplasm accessions along with four elite check clones were analysed for photosynthetic rate, stomatal conductance and changes in the level of pigments such as chlorophyll, carotenoids, anthocyanins and osmolytes (proline and glycine betaine) under water stress. The tolerant accessions showed less reduction in photosynthetic rate and stomatal conductance (g_s) compared to susceptible ones under drought stress. The photosynthetic pigments chlorophylls and carotenoids were found reduced but the reduction was negligible in the tolerant accessions. The results showed that the tolerant accessions and check clones accumulated more anthocyanins, proline and glycine betaine compared to the susceptible ones in response to water deficit stress. The higher level of proline and glycine betaine observed in tolerant accessions shows their association with drought tolerance.

KEYWORDS: *Hevea brasiliensis*, Germplasm Accessions, Drought Tolerance, Chlorophyll, Carotenoids, Anthocyanin, Proline, Glycine Betaine

Hevea brasiliensis, the major commercial source of natural rubber, is a deciduous perennial tree belonging to the family Euphorbiaceae. Its origin is in the Amazon basin of South America where a wet equatorial climate exists (Gonçalves *et al*., 2009). It is now widely cultivated in south-east Asian countries like China, Thailand, Malaysia, Indonesia, India, Sri Lanka and Vietnam. In India, the traditional rubber growing regions are Kerala state and Kanyakumari district of Tamil Nadu, where a favourable climatic condition for its cultivation exists (Thomas *et al*., 2015). The increased global demand for natural rubber and the decreased land availability in the traditional area forced its cultivation to be extended to non-traditional regions which include drought prone areas such as North Konkan regions, certain parts of Karnataka, Odisha, Madhya Pradesh and low temperature prevailing areas of north-eastern states.

In plants, the growth and development as well as the yield and productivity are greatly influenced by various environmental stress conditions. The consequence of stress perturbation is an alteration in the metabolic behaviour of the cell, leading to a cascade of molecular and biochemical events which facilitate a stable state (Radomiljac *et al.,* 2013). Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Water deficit is a major factor which affect photosynthesis and also it causes changes in the level of chlorophyll and carotenoid contents. Chlorophyll is one of the major chloroplast components for photosynthesis, and the decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation. Both chlorophyll a and b are prone to soil dehydration (Farooq *et al.,* 2009). Anthocyanins are water-soluble non photosynthetic pigments derived from flavonoids. They may be induced by various environmental factors including visible and UV-B radiation, cold temperatures, water stress etc. The subsequent production and localization of anthocyanins in root, stem and especially in leaf tissues may help the plant to develop resistance to various environmental stresses. (Chalker-Scott, 1999).

Osmotic adjustment is another major plant response to water stress which involves the accumulation of low molecular weight water-soluble compounds known as osmolytes. The most common osmolytes or compatible solutes are polyols, sugars, aminoacids like proline, and quaternary ammonium compounds like glycine betaine. These compounds play an important role in stabilizing proteins and cell membranes as well as in maintaining cell turgor (Rodziewicz *et al*., 2014). Proline accumulation has been reported in various biotic and abiotic stress conditions which include salinity, drought, high and low temperatures, UV irradiation, heavy metal, pathogen infection, nutrient deficiency and atmospheric pollution (Hare and Cress, 1997). Glycine betaine has reported to be involved in the protection of photosynthetic machinery, inhibition of ROS accumulation, membrane integrity and activation of some stress related genes (Chen *et al*., 2008).

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Also it maintains enzyme activity by the protection of quaternary structure of proteins from damaging effects of environmental stresses (Sakamoto *et al*., 2002). The tolerant or susceptible species shows varying levels of stress tolerance depending on the levels of accumulation of these compounds under different abiotic stress conditions (Giri, 2011; Chen *et al*., 2008).

Wild relatives of cultivated species remain as a potential source of drought tolerance in various crops (Shimshi *et al*., 1982). The wild accessions of *Hevea* collected from its primary centre of origin, the Amazon forests, is an excellent repository of various useful traits including drought tolerance. Significant genotypic differences for various drought related characters were noticed in *Hevea* (Sumesh *et al*., 2011) Many *Hevea* germplasm lines have already identified with moderate to good drought tolerance potential (Nair *et al*., 2011; Mercy *et al*., 2010**).** In the present study, drought tolerance potential of 10 *Hevea* germplasm accessions along with relatively drought tolerant and susceptible check clones were analysed for pigments and osmolytes and the results are discussed.

MATERIALS AND METHODS

Six month old polybag plants of 10 germplasm accessions (6 relatively drought tolerant - RO 3261, AC 612, RO 3157, RO 3184, RO1406, RO 1425 and 4 susceptible- RO 3242, MT 1619, RO 2360, AC 4084) 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 grown in glass house were subjected to water deficit stress by withholding irrigation for seven days during the summer season of 2014. A set of control plants was also maintained by irrigating on alternate days. The magnitude of impact of water stress was assessed by measuring the CO_2 assimilation rate (A) and stomatal conductance (g_s) using a portable photosynthesis system (LI-6400, LI-COR, USA). The gas exchange measurements were carried out during 8.30-11.00 IST at a fixed CO_2 concentration of 400 ppm and photosynthetically active radiation of 500µ mol $m^{-2}s^{-1}$ using a leaf chamber light source, attached to LI-6400.

Leaf samples were collected on the seventh day of drought imposition for biochemical analyses. Physiologically mature leaves were collected from the selected plants (n=4) for the estimation of chlorophyll *a*, chlorophyll *b*, carotenoids, anthocyanins, proline and glycine betaine. Cholorophyll was estimated by the method of Arnon (1949) and the carotenoids were estimated according to Lichtenthaler (1987). Anthocyanins were estimated according to Wanger (1979). Free proline content in the leaves was determined following the method of Bates *et al.* (1973). Glycine betaine estimation was done in dried leaf powder as per the method of Grieve and Grattan (1983). All data were analyzed statistically using ANOVA with IBM-SPSS analytical software package version 16.0 (IBM Corporation, USA).

RESULTS AND DISCUSSION

The germplasm accessions for the study have been selected based on our previous study (Thomas *et al*., 2015). The extent of drought stress was assessed by measurement of CO_2 assimilation rate (A) and stomatal conductance (g_s) and the results are given in Fig. 1 and 2. The control plants maintained a higher rate of CO_2 assimilation rate (A) in tolerant germplasm accessions RO3261 and AC 612 as well as in tolerant check clones RRIM 600 and RRII 430 compared to susceptible ones (Fig. 1). After seven days of drought stress imposition CO_2 assimilation A has been reduced in all plants and the decline was found high in the susceptible accession RO 3242 (0.188 µ mol m^{-2} s^{-1}) followed by RRII 105 (0.37 µ mol m^{-2} s^{-1}) and RRII 414 (0.51µ mol m^{-2} s^{-1}). Stomatal conductance (g_s) was reduced near to zero in susceptible germplasm accessions RO 3242, MT 1619, RO 2360 and susceptible check clones RRII 105 and RRII 414 (Fig. 2). The most important physiological parameter being affected by drought or water deficit is photosynthesis; causing a progressive and severe reduction in the CO_2 assimilation capacity. This decrease in net photosynthetic rate is associated to stomatal closure induced by a decline in leaf cell turgor that limits diffusion of CO_2 into the substomatal chamber. Under such conditions, photoinhibition may occur, a process that reduces quantum yield of PSII and also it induces photorespiration and H_2O_2 production (Hossain *et al.,* 2009).
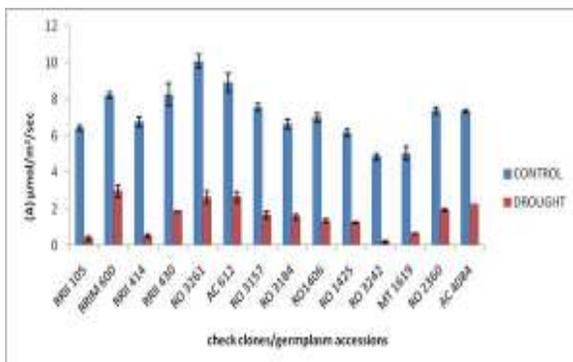
Figure1. CO$_2$ assimilation rates (A) in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days.
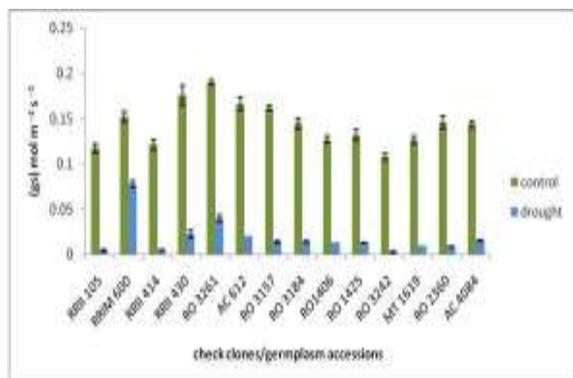

Figure 2. Stomatal conductance (gs) in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days.

The level of chlorophyll *a*, chlorophyll *b,* total chlorophyll and carotenoids were found to be reduced under drought stress in all plants (Fig. 3,4,5 and 6). The results shows that under drought stress the level of chlorophyll content is only slightly reduced, maintaining values near to the control level which gives an indication that chlorophyll degradation is a delayed process under drought stress compared to rate of photosynthesis and stomatal conductance (g$_s$). Decreased chlorophyll under water stress generally occurs due to damage of chloroplasts caused by oxidative bursts or due to changed ratios of lipid protein complexes or elevated chlorophyllase activity which degrades chlorophyll and damages light harvesting machinery (Kaya *et al.,* 2006). The level of accessory pigment carotenoids also maintained a value near to control. The clone RRIM 600 maintained higher level of carotenoids among the clones/accessions studied. The level of anthocyanin

showed an increase under drought stress (Fig. 7). The tolerant germplasm accession RO3261 showed the highest level of anthocyanin content.
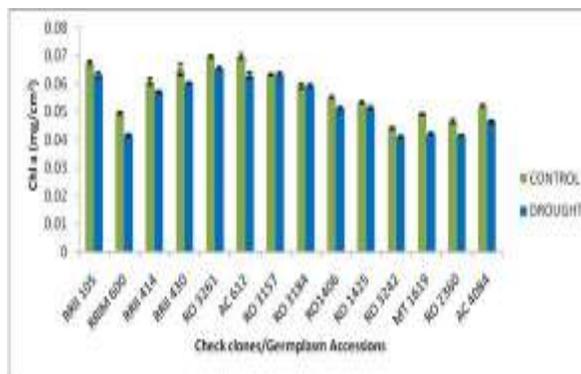

Figure 3. Chlorophyll a content in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days.
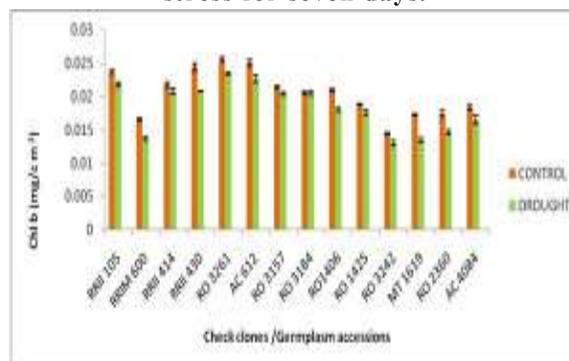
Figure 4. Chlorophyll b content in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days.
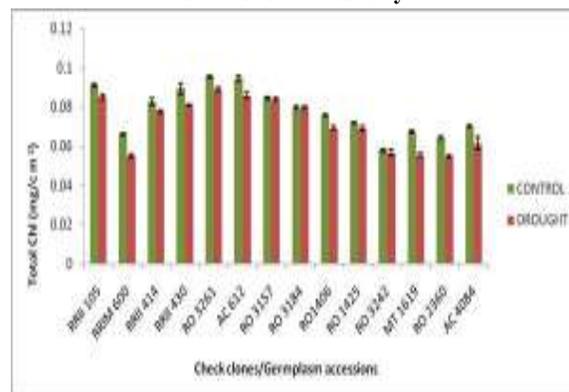

Figure 5. Total Chlorophyll content in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days
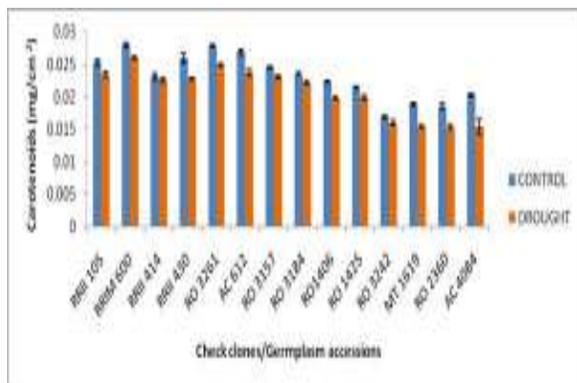
Figure 6. Carotenoids content in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days

Figure 7. Anthocyanin content in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days

Free proline content in leaves of different *Hevea* genotypes is given in Fig. 8. The free proline content in leaves under controlled condition was in the range of 59 to 144 µg/ g FW. All the germplasm accessions showed significantly higher level of proline content compared to check clones. Under drought stress, a tremendous increase has been observed in the level of proline which comes in the range of 450 to 760 µg/ g FW. The percentage of increase has been found high in all the tolerant germplasm accessions on comparison with susceptible accessions, but a markable increase was not observed in tolerant (RRIM 600 and RRII 430) as well as susceptible check clones (RRII 105 and RRII 414). Here a positive correlation can be observed between drought stress tolerance and proline accumulation in the germplasm accessions. Proline is an important parameter to measure the stress tolerance capacity of the plants and its accumulation is considered as an early response to drought stress (Ramanjulu and Sudhakar, 2000). The level of proline accumulation in

plants can be 100 times greater than in control condition and it differs from species to species (Verbruggen *et al.*, 2008). It protects the plants under different stress conditions and also helps plants to recover from stress more rapidly (Hayat *et al.*, 2012). Proline accumulation normally occurs in cytoplasm where it functions as molecular chaperons stabilizing the structure of proteins and its accumulation buffers cytosolic pH and maintains cell redox status. It has been suggested that its accumulation may be part of a stress signal influencing adaptive responses (Ashraf and Foolad, 2007; Hayat *et al.*, 2012).
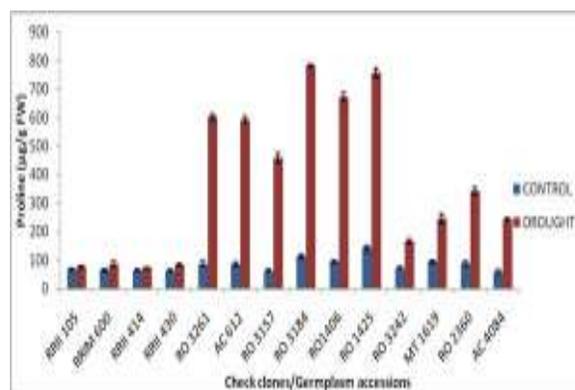

Figure 8. Free proline content in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days.

The level of accumulation of glycine betaine was also found to be increased on drought stress imposition in different *Hevea* clones and accessions (Fig. 9). In tolerant check clones and germplasm accessions, percentage of increase was high, when compared to susceptible check clones and germplasm accessions. The tolerant check clones (RRIM 600 and RRII 430) showed percentage increase of 31 and 35 respectively. Among the germplasm accessions, 27% increase was observed in the tolerant accession RO 3184. The percentage increase was found very less in the susceptible accession MT 1619 (1.2%). All the tolerant accessions showed a percentage increase in the range of 14% to 27%. In plants, under water stress, glycine betaine is abundant in chloroplast and plays a vital role in protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Genard *et al.*, 1991). The glycine betaine content increased under drought stress in barley, spinach, maize, sugar beet, and in higher plants (Bohnert *et al.*, 1995; Nakamura *et al.,* 2001). Reactive oxygen species (ROS) production is enhanced under abiotic stresses which lead to photoinhibition of PSII in

chloroplast. Glycine betaine protects the photosynthesis machinery by stabilizing the activity of repair proteins and thus provides tolerance to abiotic stresses even at low concentration (Takahashi *et al.,* 2008).
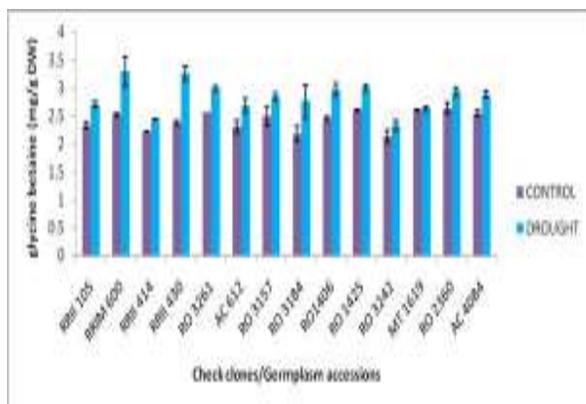
Figure 9. Glycine betaine content in different *Hevea* germplasm accessions and check clones under control and after imposition of drought stress for seven days.

CONCLUSION

The results indicated that the accumulation of anthocyanin, proline and glycine betaine were high in the tolerant *Hevea* accessions compared to the susceptible ones in response to water deficit stress. The photosynthetic pigments Chlorophyll a, Chlorophyll b, total chlorophyll and carotenoids were found reduced but the reduction was negligible. The rate of photosynthesis was also found be higher in the tolerant accessions which clearly show a positive correlation between drought tolerance and osmolyte accumulation. The differential level of accumulation of the important osmolyte, proline in mesophyll cells of relatively drought tolerant *Hevea* accessions are noteworthy to explore further. It is clearly demonstrated that higher accumulation of osmolytes like proline and glycine betaine imparted drought tolerance potential in the germplasm lines. The accumulation of non photosynthetic pigment anthocyanin may also help to develop tolerance to drought stress.

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