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Biochemical Evaluation of RRII 400 Series Clones of *Hevea Brasiliensis* for Drought Tolerance

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***Hevea brasiliensis* is the most important commercial source of natural rubber (NR). Its cultivation is being extended to drought prone non-traditional areas to meet the increasing global demand in almost all the NR producing countries.To identify suitable drought tolerant clones for establishment in such areas, five modern *Hevea brasiliensis* clones (RRII 400 series) were evaluated along with the most popular clone in India, RRII 105 and a known drought tolerant check clone, RRIM 600.The drought tolerance potential of these clones were evaluated using physiological and biochemical parameters such as, leaf wax content, antioxidants, antioxidant enzyme activities and gas exchange parameters. Leaf epicuticular wax content, an important trait associated with drought and heat tolerance was found to be significantly higher in clone RRII 430 than in other 400 series clones and RRII 105. The clone RRII 430 also had significantly higher levels of ascorbic acid (antioxidant) content and super oxide dismutase (SOD) activity and those components play vital roles in defense against oxidative stress.When subjected to drought stress, this clone showed better net photosynthesis rate (P_N) and stomatal conductance (g_s) compared to other 400 series clones. The results indicated that the modern *Hevea* clone RRII 430 is the most potential drought tolerant clone among the 400 series clones.**

Key words: *Hevea brasiliensis*, drought tolerance, leaf epicuticular wax, ascorbic acid, SOD activity

Hevea brasiliensis is the only genus that is grown commercially as the source of natural rubber in India. Rubber plants are traditionally grown under rainfed conditions. But due to increasing demand for natural rubber, the cultivation is being extended to less and marginally suitable areas. Soil and atmospheric drought and high temperature are the major environmental factors limiting its growth and yield in such areas (1). Water stress is one of the most important environmental factors inhibiting photosynthesis (2). Drought stress concomitant with high intensity of sunlight inflict damage to the green leaves resulting in severe inhibition of photosynthesis (3). Several physiological traits are adversely affected due to drought stress in *Hevea* (4, 5, 6). Plants utilize several protective mechanisms to maintain normal cellular metabolism and prevent damage to the structure and function of cellular components. Changes in the activities of enzymes *viz.* superoxide dismutase (SOD), peroxidase *etc.* and accumulation of antioxidants such as ascorbic acid and glutathione have been reported in several crops to overcome the deleterious effects of oxidative stress induced by environmental stress (7,8). Accumulation of epicuticular wax content on the leaf surface is reported to be an important parameter associated with drought and heat tolerance (9, 10). Sreelatha *et al.* (11) observed that a high rate of leaf peroxidase activity is generally associated with drought tolerance in *Hevea*.

Significant genotypic differences for various drought related characters were reported in *Hevea* (12,5). The ability of a plant to withstand water deficit is associated with numerous inherent traits that contribute to drought tolerance. An understanding of these characters which are more directly related to drought tolerance helps in easy identification of genotypes suitable for drought prone areas. RRII 400 series clones are the recently released clones of Rubber Research Institute of India and their drought tolerance potentials are largely unknown. In the present study five RRII 400 series clones which showed better growth and yield performance than RRII 105, the most popular high yielding clone of India, were subjected to soil moisture deficit stress for seven days and various biochemical parameters associated with drought tolerance traits were analyzed. The results are discussed in terms of identifying the most potential drought tolerant clone among the RRII 400 series.

Materials and Methods

Seven - month-old plants of five RRII 400 series clones (RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430)

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along with plants of RRII 105 (drought sensitive) and RRIM 600 (relatively drought tolerant) grown in big polythene bags (size 65 x 35 cm) were kept in the open field at the Rubber Research Institute of India campus during summer 2011. The plants (n=6) were subjected to soil water deficit stress by withholding irrigation for 7 days. There was no rainfall during the experimental period and the environment was dry with 8.0 hours of mean sunshine per day. The minimum and maximum temperatures were 21.0° C and 34.4° C respectively. The magnitude of impact of water stress was assessed by measuring leaf water status and photosynthesis. Mid-day leaf water potential (øL) was measured at 11.30 am using a dew point micro-voltmeter connected with C-52 sample chambers using Psypro water potential system (Wescor, USA). The net photosynthetic rate (P_N) and stomatal conductance (g_s) were measured using a portable photosynthesis system (LI-6400, LI-COR, USA). The observations were recorded during 8.30-11.00 IST at a fixed CO_2 concentration of 400 ppm and photosynthetically active radiation of 500 µmol $m^{-2}s^{-1}$ using a leaf chamber light source, attached to Li-6400.

Biochemical analysis: Leaf samples were collected for biochemical analyses on the seventh day of drought imposition. The leaf epicuticular wax content was determined according to the method described by Ebercon *et al.* (13). The colorimetric method is based on the colour change produced due to the reaction of wax with acidic $K_2Cr_2O_7$. For the extraction of ascorbic acid and thiols, frozen leaf tissue (0.25 g) was ground using liquid N_2 and homogenized in 1.5 mL 5% sulphosalicylic acid. The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4°C. The ascorbic acid content in the supernatant was assayed according to Kampfenkel *et al.* (14). The thiol content was estimated according to Boyne and Ellman (15). For the assay of enzymes, frozen leaf tissues (0.4 g) were powdered using liquid N_2 and homogenized in 3 mL ice-cold potassium phosphate buffer (50mM, pH 7.4) containing 1% (w/v) polyvinyl pyrrolidone. The homogenates were centrifuged at 15,000 rpm for 20 minutes at 4°C and the supernatants were used as source of enzyme assays. The peroxidase activity was assayed according to the method described by Guilbault (16) and expressed as change in OD/min/mg protein. Total SOD activity was assayed by the inhibition of the photochemical reduction of Nitro blue tetrazolium (NBT). One unit of SOD was defined as the amount of enzyme (mg protein) that produced 50 per cent reduction of NBT under the assay conditions as described by Giannopolitis and Ries (17). The whole set of experiment was repeated again for confirmation and the data were analysed statistically by analysis of variance (ANOVA) using Cropstat V.7.2 software.

Results and Discussion

To analyze the responses of young plants to drought stress and to find out drought tolerant clones, specific parameters associated with drought tolerance traits were studied in RRII 400 series *Hevea* clones after exposing them to water deficit stress for one week period. T impact of water stress on these plants was assessed using leaf water potential and gas exchange measurements. Mid-day leaf water potential data showed significant differences among the *Hevea* clones after seven days of drought imposition (Fig. 1). Clone RRII 430 maintained better leaf water potential significantly at par with clone RRIM 600 after seven days of drought; whereas, clones RRII 414 and RRII 105 could not maintain better leaf water potential and showed more negative values.

The data on photosynthesis rate (P_N) and stomatal conductance (g_s) of different *Hevea* clones are shown in Fig. 2 and 3. The photosynthetic rate under irrigated conditions varied from 9.4 to 12.0 µmol m^{-2} s^{-1} and under

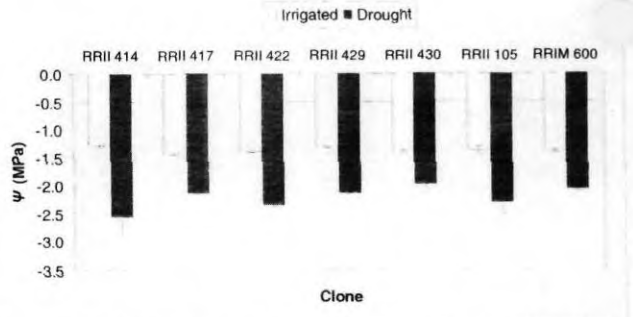

Fig. 1: Midday leaf water potential (ψ) of *H. brasiliensis* clones after drought imposition for seven days. The ψ values for drought treatment (shaded) are significantly different from irrigated (unshaded) plants at P=0.05 in all the clones. CD at P=0.05 for drought (0.30) and non-significant for irrigated condition.

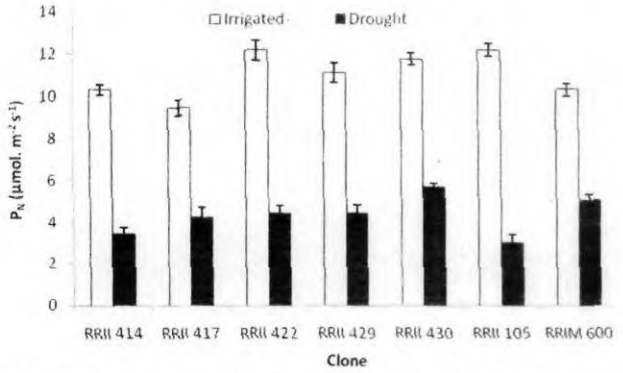

Fig. 2: Net photosynthetic rate (P_N) of *H. brasiliensis* clones after drought imposition for seven days. The P_N values for drought treatment (shaded) are significantly different from irrigated (unshaded) plants at P=0.05 in all the clones. CD at P=0.05 for irrigated (1.00); drought (0.94).

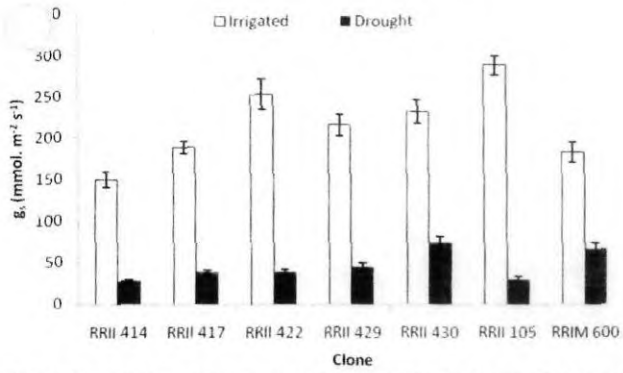

Fig. 3: Stomatal conductance (g_s) of *H. brasiliensis* clones after drought imposition for seven days. The g_s values for drought treatment (shaded) are significantly different from irrigated (unshaded) plants at P=0.05 in all the clones. CD at P=0.05 for irrigated (35.8); drought (13.6).

drought conditions it varied from 3.0 to 5.7 µmol $m^{-2}s^{-1}$. In general, photosynthetic rate and stomatal conductance significantly decreased during drought s[illegible]s compared to irrigated control in all the clones. There were significant variations among the clones in these parameters. Under drought stress, clone RRII 430 showed significantly better P_N and g_s than other RRII 400 series clones. Under stress-free conditions also this clone recorded the maximum P_N and g_s. Sumesh *et al.* (6) observed similar results in these clones in an earlier study. The popular high yielding clone RRII 105 recorded the least 'P_N' under drought conditions (Fig. 2). This clone was already reported to be a drought susceptible clone (18). The relatively drought tolerant clone, RRIM 600 showed similar results for photosynthesis rate and stomatal conductance as RRII 430. The clone RRIM 600 was characterized well for its drought tolerance traits including photosynthetic gas exchange and fluorescence parameters (19, 6). This clone was proven to be established well in severe drought prone regions like North Konkan and other Central India regions (3).

In the present study stomatal conductance was drastically inhibited in young plants of *Hevea*. Indeed, there were significant clonal differences among the population studied. The reduction was severe in clones such as RRII 414, RRII 417, RRII 422 and RRII 105, where as clones like RRII 430 and RRIM 600 showed better conductance (Fig. 3). It has been reported earlier that stomatal closure is one of the first responses to soil moisture deficit in crop species (20). When water loss from leaves exceeds the uptake, stomatal conductance decreases and the limitation of CO_2 diffusion results in substantial reduction in photosynthesis (21). Earlier workers reported clonal variations in the effect of water stress on photosynthesis in *Hevea* (22, 5, 6).

The data on leaf epicuticular wax content of different *Hevea* clones under drought stress is given in Table 1. Significant variations were observed for epicuticular wax content in different *Hevea* clones. Among the clones, RRII 430 recorded significantly higher epicuticular wax content than other RRII 400 series clones and RRII 105. The wax content in RRIM 600 was on par with RRII 430. Epicuticular waxes consist of a variety of long-chain aliphatic compounds with different functional groups. The hydrophobic properties of these compounds reduce water loss via transpiration to the atmosphere (23). Positive correlation of leaf epicuticular wax content with drought tolerance has been observed in several crops (10, 24). Presence of epicuticular wax on the leaf surface of rubber plants helps in reducing cuticular and stomatal transpiration and promotes reflection of radiant energy by canopies (9).

The content of ascorbic acid and thiols and activity of peroxidase and superoxide dismutase in the leaves of different *Hevea* clones is given in Table 1. The clone RRII 430 showed significantly higher levels of ascorbic acid content and super oxide dismutase activity in leaves than other 400 series clones under drought stress conditions. Drought and associated high intensity of light lead to diversion of photosynthetic electrons resulting in the production of active oxygen species (AOS). However,

Table 1: Epicuticular wax, thiols, ascorbic acid, peroxidase activity and superoxide dismutase activity in the leaves of different *Hevea* clones after imposition of drought stress for seven days

Clone	Epicuticular wax ($\mu g/cm^2$)	Thiols (mg/g)	Ascorbic acid (mg/ g)	Peroxidase (OD/min/mg protein)	Super oxide dismutase (Units/mg protein)
RRII 414	61.08±2.33	5.22±0.31	2.87±0.21	0.74±0.15	4.41±0.14
RRII 417	62.96±1.63	4.62±0.29	2.63±0.21	0.93±0.19	4.49±0.12
RRII 422	59.95±1.63	3.25±0.28	1.46±0.05	1.91±0.11	4.38±0.09
RRII 429	61.84±1.88	2.84±0.30	2.37±0.22	1.47±0.22	4.42±0.08
RRII 430	68.99±1.23	4.01±0.16	3.60±0.28	1.06±0.07	5.50±0.31
RRII 105	61.83±0.72	3.16±0.52	1.63±0.08	0.72±0.02	3.64±0.30
RRIM 600	68.24±1.99	3.85±0.20	3.14±0.05	1.03±0.15	5.47±0.17
CD($P \leq 0.05$)	5.00	0.91	0.54	0.43	0.57

The values are mean ± SE (n=6)

a highly efficient antioxidant defence system consisting of both enzymatic and non-enzymatic antioxidants is present in plants to detoxify the harmful AOS (25, 11). The superoxide radical (O_2^-) is mainly scavenged by the enzyme SOD which may play a critical role in preventing oxidative damage. The increase in SOD activities induced by water stress was more rapid and greater in drought tolerant than in sensitive barley (26) and tomato cultivars (27). *Hevea* plants over expressed with MnSOD showed less reduction in photosynthesis under water stress (28). Ascorbate is an important antioxidant and the ascorbate/ dehydroascorbate ratio is a good indicator of stress experienced by plants (14). Ascorbic acid eliminates reactive oxygen species through multiple mechanisms and also maintains membrane-bound antioxidant á-tocopherol in the reduced state (29).

Plant responses to water deficit stress are complex phenomena and encompass many aspects including stress sensing and signaling, changes in growth and biomass allocation patterns, water status homeostasis, decreased stomatal conductance and CO_2 assimilation, osmoregulation and detoxification processes (30). The ability of a plant to withstand drought stress depends on numerous traits associated with modulation of chloroplast and cytoplasm composition and activities. Among the clones, RRII 430 recorded significantly higher leaf epicuticular wax content than other RRII 400 series clones. This clone also had significantly higher levels of ascorbic acid content and super oxide dismutase activity that plays vital role in defense against oxidative stress. Under drought stress, this clone showed better photosynthesis rate and stomatal conductance. The results indicated that the *Hevea* clone RRII 430 is the most potential drought tolerant clone among the recently released RRII 400 series clones.

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