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Effects of drought stress on chlorophyll *a* fluorescence in two rubber tree clones

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A R T I C L E I N F O

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A B S T R A C T

This study compared the photosynthetic responses of two clones (RRIM600 and FX3864) of the rubber tree (*Hevea brasiliensis* L.) subjected to drought stress. This stress was imposed by complete removal of watering until the leaf water potential (Ψ_w) reached critical values, which occurred at 38 days after water deficit (DAWD) for both rubber clones. One-year-old rubber plants were cultivated in 20 L pots filled with a soil:sand:humus ratio of 4:3:3. Photosynthetic performance was analyzed by measuring chlorophyll *a* fluorescence transients at 0, 28, 36, and 38 DAWD. The photochemical process was altered to some extent following water deficit. A decrease in Ψ_w was observed for both rubber clones at 36 DAWD. The FX3864 clone was more susceptible to drought and showed a deficiency in photosynthetic electron transport. The RRIM600 rubber clone showed an advantage over the FX3864 clone in terms of stability and efficiency in utilizing energy in low rainfall sites.

1. Introduction

Low water availability is the major environmental factor limiting growth, development, and the agricultural production of plants worldwide (Silva et al., 2013). An estimated one-third of the world's terrestrial area suffers from water stress, which is predicted to increase owing to global warming, enhancing the reduction in crop production in many key production regions (Tack et al., 2015).

Physiologically, several plant processes are negatively affected by water stress. These effects occur through osmotic stress and different biochemical responses in plants such as cell turgidity, stomatal conductance, transpiration, photosynthesis, respiration, antioxidant activity, and light absorption and capture, resulting in reduced crop production (Lawlor and Cornic, 2002; Hsiao et al., 2010; Silva et al., 2013; Velázquez-Márquez et al., 2015). According to Chaves et al. (2002), the effects of low water availability on plant physiological processes are influenced by both the intensity and duration of the environmental stress as well as the genetic capacity of the genotype/species to cope with stress. Thus, the maintenance of vital plant metabolism functions as well as the rapid recovery of water status after rehydration are required for crop tolerance to low water availability (Waseem et al., 2011).

Chlorophyll *a* fluorescence (CF) has been used to evaluate the extent of damage to the photosynthetic apparatus, particularly to photosystem II (PSII), under several types of environmental stress because the technique is a non-destructive, simple, and rapid testing method (Mehta et al., 2010; Zushi and Matsuzoe, 2017). Thus, CF is a useful tool for monitoring and screening the stress tolerance of plant species and genotypes (Gonçalves et al., 2010).

Under drought stress, damage to PSII reaction centers (RCs) inhibits the primary photochemistry affecting the photosynthetic electron transport process from CRs to the quinone A (Q_A), quinone B (Q_B), and plastoquinone (PQ) pools (Mehta et al., 2010; Zushi and Matsuzoe, 2017). Previous work has described decreases in the maximum quantum yield of PSII (F_v/F_m) and differences in both energetic connectivity between PSII units (L-band) and stability of the oxygen evolution complex (OEC) (K-band) in barley (*Hordeum vulgare* L.), passion fruit (*Passiflora edulis* Sims) and cowpea (*Vigna unguiculata*) cultivars differing in drought tolerance (Souza et al., 2004; Oukarroum et al., 2007; Gomes et al., 2012). These studies demonstrated that after re-watering, signals of recovery are observed owing to an increase in drought resistance. These results suggest that it is possible to differentiate the tolerance of genotypes to drought stress at the level of PSII. Therefore, the use of drought tolerant genotypes is a viable alternative

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to increase plant productivity in water-deficient environments (Waseem et al., 2011).

The rubber tree (*Hevea brasiliensis* L.) is a Euphorbiaceae native to Amazônia (Brazil), a tropical region. Rubber trees have been cultivated in all other Brazilian regions and in several countries around the world. However, the climatic conditions of most of the new cultivation regions show reduced rainfall during certain periods of the year (Macedo et al., 2002). Therefore, the maximal agricultural production of rubber trees has only been made possible through irrigation practices. However, because water availability can be limited during certain periods of the year due to low rainfall, the use of drought-tolerant genotypes is a viable alternative to improve productivity. The objective of this study was to compare the photosynthetic changes of two clones (RRIM600 and FX3864) of the rubber tree (*H. brasiliensis*) subjected to water stress, through analysis of transient fluorescence kinetics for chlorophyll *a*.

2. Materials and methods

2.1. Plants, growth conditions, and drought treatment

The experiment was performed using seedlings of two rubber tree (*Hevea brasiliensis* L.) clones, RRIM600 and FX3864, which are the most commonly cultivated genotypes in Brazil. One-year-old rubber seedlings, approximately 50 cm tall with 10 leaves, were obtained from a commercial nursery (Linhares, Espírito Santo State, Brazil) and cultivated in 20-L pots (one plant per pot) filled with a soil:sand:humus ratio of 4:3:3. For 30 days, the pots were maintained under greenhouse conditions with an average midday photosynthetic photon flux (PPF) of 800 µmol (photons) $m^{-2}s^{-1}$. Next, two different irrigation regimes were applied to each rubber clone: 25 plants were maintained under water-deficit (non-irrigated plants) and 13 plants were maintained in well-watered conditions (plants were irrigated daily and used as a control).

Because rubber trees show fall foliar when maintained under water deficit, a higher number of plants were used for the water deficit treatment to ensure sufficient CF and leaf water potential (Ψ_w) measurements, which was made on 12 and five randomly selected plants/treatment, respectively. Drought stress was imposed by completely removing irrigation. The plants remained under persistent drought for 38 days until the Ψ_w values were sufficiently negative (approximately −2.0 MPa).

2.2. Chlorophyll a fluorescence

Transient chlorophyll *a* fluorescence was measured at 0, 28, 36, and 38 DAWD, using a Plant Efficiency Analyzer (Handy-PEA, Hansatech, King's Lynn, Northfolk, England, UK). The measurements were made on 12 leaves (third or fourth leaf from the apex) that were dark-adapted for 30 min using a leaf clip (Hansatech). The light intensity reaching the leaf was 3000 µmol (photons) $m^{-2}s^{-1}$, which was sufficient to generate maximal fluorescence for all treatments. The fast fluorescence kinetics (F_0 to F_m) was recorded from 10 µs to 1 s. The fluorescence intensity at 20 µs (considered F_0), 100 µs, 300 µs, 2 ms (F_J), and 30 ms (F_I), and maximum fluorescence (F_m) were recorded and analyzed according to the JIP-test (Strasser et al., 2004; Stibert and Govindjee, 2011).

2.3. Leaf water potential (Ψ_w)

The predawn xylem water tension (MPa) was measured at 0, 28, 36, and 38 days with a Scholander-type pressure chamber (Scholander et al., 1965) in five plants (one leaf per plant) per treatment. When necessary, latex extravasated by petiole was removed using a paper towel.

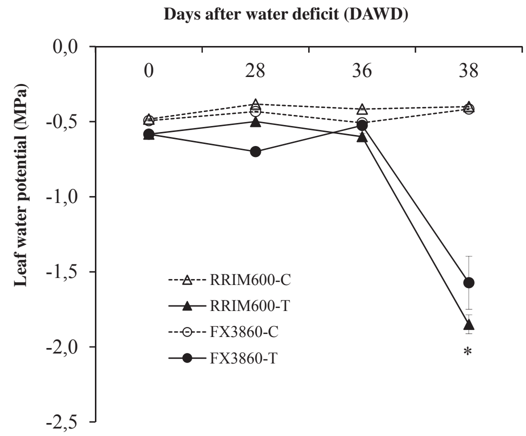

Fig. 1. Leaf water potential (*Ψw*) of two rubber genotypes RRIM600 and FX3864 submitted to an imposed water stress during 38 days. The data shows value ± standard errors. Values are means for five plants. * indicate significative difference ($p \leq 0.05$) between clones. C – control, T – treatment (drought stress).

2.4. Statistical analyses

A completely randomized design in a factorial scheme was adopted [two water treatments (water deficit and well-watered) × four time points (0, 28, 36, and 38 days)] for each clone. Microsoft Excel 2007 was used to create graphs for the OJIP transients. Leaf water potential and JIP-test parameters were evaluated through variance analysis. Means and standard errors (± S.E.) were reported with statistical significance ($p < 0.05$) determined by Tukey's test.

3. Results

3.1. Leaf water potential (Ψ_w)

Decrease in leaf water potential (Ψ_w) was evident after 36 DAWD (Fig. 1). However, a significant difference between rubber clones was only observed at 38 DAWD (−1.85 and −1.57 MPa for RRIM600 and FX3864, respectively) (Fig. 1).

3.2. Chlorophyll a fluorescence

Relative to the control, an increase in the J-step fluorescence level was the most evident characteristic of CF and OJIP transients for both rubber tree clones following water deficit. In both clones, the J-step fluorescence levels increased over time with water deprivation (Fig. 2a and b). Although an increase in J-step fluorescence levels in RRIM600 relative to control was observed at 28, 36, and 38 DAWD, no difference in J-step fluorescence levels was observed at 28 and 36 DAWD for the FX3864 rubber clone (Fig. 2a and b).

To further elucidate the differences between clones in response to drought stress, the relative fluorescence between the steps O and K [20 and 300 µs, respectively = $V_{OK} = (F_t - F_0)/(F_K - F_0)$] and O and J [20 µs and 2 ms, respectively = $V_{OJ} = (F_t - F_0)/(F_J - F_0)$] were normalized and are shown as the kinetic difference $\Delta V_{OK} = V_{OK(treatment)} - V_{OK(control)}$ and $\Delta V_{OJ} = V_{OJ(treatment)} - V_{OJ(control)}$, respectively (Fig. 2c–f). The kinetic differences ΔV_{OK} and ΔV_{OJ} make the L- and K-bands visible, respectively. These bands have a peak around 0.15 and 0.3 ms, respectively. The L and K-band are an indicator of energetic connectivity or grouping between PSII units and the stability of OEC, respectively (Strasser and Stirbet, 1998). In this study, the response of the L- and K-bands to water deficit was different for two rubber clones. In RRIM600, the energetic connectivity and the stability of OEC decreased from 28 DAWD and reached a maximum decrease at 38 DAWD (Fig. 2c and e). In FX3864, the appearance of positive L- and K-bands occurred only at 36 DAWD (Fig. 2d and f). Notably, at 38 DAWD, the maximum values for both the L- and K-bands were higher for