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Photochemical activity in *Dalbergia nigra* (Vell.) Fr. Allem. seedlings submitted to water deficit

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Abstract

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In this study, we evaluated the influence of the daily variation of both intensity of photosynthetically active radiation (PAR) and temperature on chlorophyll *a* fluorescence parameters in Jacarandá-da-Bahia seedlings submitted to two treatments: T1 (control) and T2 (water deficit during seven days). It was used seedling with approximately 90 days old cultivated in plastic pots contend soil. After 45 days of acclimation, the treatments were applied. In T2, the water deficit was applied by total suspension of irrigation. The physiological evaluations were made a long of the day (from 6 a.m. to 6 p.m.). The water deficit caused a significant reduction of the maximal fluorescence and in area below the fluorescence curve. Furthermore, the water deficit improved the effects of the high temperature and PAR on the energetic connectivity (or grouping between photosystem II units) and of oxygen-evolving complex (OEC) stability, evidenced by the high and positive values of L-band and K-band in those daytimes with higher incidence. Finally, the effects of water deficit were more evident on the OEC stability.

Keywords: oxygen-evolving complex; L-band; K-band; chlorophyll *a* fluorescence; reforestation

Since the Portuguese colonization occurred in the 16th century up to the present, environmental degradation of Brazilian ecosystems has been growing consistently. Nowadays, projects aiming to restore those areas degraded by the productive sector have been highly prized. However, the success of a restoration project is dependent of fundamental criteria adopted, among which the knowledge about the tolerance to changes in water availability of the plant species (Martins 2009; Matos et al. 2018). Soil water stress is the major abiotic factors limiting plant growth and development, resulting in enormous losses in both agronomic and forest productivity (Paiva et al. 2005; Gomes et al. 2012; Wang et al. 2015).

In vivo chlorophyll *a* fluorescence is a technique that has been largely used to evaluate the plant physiological responses to water deficit. According to Mehta et al. (2010) and Zivcak et al. (2013), *in vivo* chlorophyll *a* fluorescence is simple, reliable, noninvasive and highly sensitive technique, allowing evaluating physiological and structural alterations in the photosynthetic apparatus in plants growing under stress. Thus, the technique plays a pivotal role in studies monitoring and screening the drought-stress tolerance of plant species and genotypes. Differences in the energetic conductivity between the photosystem II (PSII) units and the oxygen-evolving complex (OEC) associated with the PSII, evaluated through of K- and L-band, respectively, can be observed in situations where the photosynthetic apparatus responds positively or negatively to drought stress, as observed by Oukarroum et al. (2007), studying differential tolerance in barley (*Hordeum vulgare* Linnaeus) cultivars. In one-year-old seedlings of rubber tree (*Hevea brasiliensis* Linnaeus), positive deviations of the L- and K-bands were observed 28 and 36 days after water deprivation to the RRIM600 and FX3864 rubber varieties, respectively, evidencing a higher tolerance of the photosynthetic apparatus in FX3864 (Falqueto et al. 2017).

Previous studies have shown that water deficit combined to high temperatures reduce the photochemical efficiency of PSII resulting in photoinhibition, as described by Lemos-Filho (2000) and Lu et al. (2003). In addition, Dias and Marenco (2007) analysed the effects of high photosynthetically active radiation (PAR) and high temperature on the photosynthetic activity of both mahogany (*Swietenia macrophylla* King) and black manwood (*Minquartia guianensis* Aublet). Under temperatures of up to 38°C, high PAR determined the photoinhibition severity of the photosynthesis. In addition, increases of the initial fluorescence (F_0) values in moderate PAR and temperatures occurred, which was associated with the faster recovery from photoinhibition.

Brazilian rosewood (*Dalbergia nigra* (Vellozo) Fr. Allemão) is a species endemic of the Atlantic rainforest and occurs in the states of Bahia and São Paulo (Carvalho 2003) and, because of its high regeneration rates in altered environments, it has a high potential to be used in projects of forest restoration (Rêgo, Possamai 2003). Within this context, the present work aimed at evaluating the influence of daily variation in the PAR and temperature on the photosynthetic parameters in seedlings of Brazilian rosewood submitted to water deficit.

MATERIAL AND METHODS

Growth conditions and drought treatment. The experiment was conducted in the greenhouse conditions of the Laboratório de Ecofisiologia Vegetal, Departamento de Ciências Agrárias e Biológicas, Universidade Federal do Espírito Santo. Three-months-old jacaranda-da-Bahia seedlings, with approximately 50 cm tall with 10 leaves, were transplanted in 3-L pots (one plant per pot) filled with a soil classified as medium/clayey texture yellow argisol (Embrapa. Centro Nacional de Pesquisa de Solos 2006).

During 45 days, the pots were maintained under greenhouse conditions with an average midday PAR of 800 μmol (photons)·m^{-2}·s^{-1} (measured as described below). After, two different irrigation regimes (treatments) were applied: plants maintained under well-watered conditions (plants were irrigated daily whit 200 ml water, treatment 1 – T1) and plants maintained under water-deficit (non-irrigated plants, treatment 2 – T2). Physiological evaluations were made in leaflets located on the medium region of the third pair of leaves from the apex, every two hours along of the day. The first measurement was performed at 6 a.m. and the last at 6 p.m.

Leaf relative water content (RWC), soil moisture and intensity of PAR. RWC was calculated according to Távora et al. (2001) with modifications. Six leaf discs (5 mm of diameter) were extracted of each plant, immediately weighted together in an analytical balance (Bioprecisa FA-2104N), obtaining the fresh mass (FM) of the tissue. The turgid mass (TM) was obtained after the hydration of the leaf discs through of submersion for 24 h in Petri dishes contend distilled water. Dry mass (DM) was obtained after drying the leaf discs during 72 h under 65°C. RWC was calculated according to the following expression (Eq. 1) and the results expressed in percentage:

$$\mathrm{RWC} = \left[\frac{\mathrm{FM} - \mathrm{DM}}{\mathrm{TM} - \mathrm{DM}}\right] \times 100 \qquad (1)$$

Soil moisture (m^3·m^{-3}) was monitored with a humidity sensor (model PC-1; Decagon Devices, USA), installed in the centre of the pots, at approximately 10 cm depth. A quantum sensor (LI-250A; LI-COR, USA) and analogical thermometers (Incoterm) were used to determine PAR and temperature, respectively.

Chlorophyll *a* fluorescence transient. The Chlorophyll *a* fluorescence was measured using a portable Plant Efficiency Analyser (Handy-PEA, Hansatech Instruments, UK), on dark-adapted for 30 min using a leaf clip (Hansatech Instruments, UK). The light intensity reaching the leaf was 3,000 μmol (photons)·m^{-2}·s^{-1}, which was sufficient to generate maximal fluorescence (F_m) for all treatments. The fluorescence signals were registered between F_0 to F_m from 10 μs for 1 s. The fluorescence intensity at 50 μs (considered as F_0), 2 ms (F_J), and 30 ms (F_I),

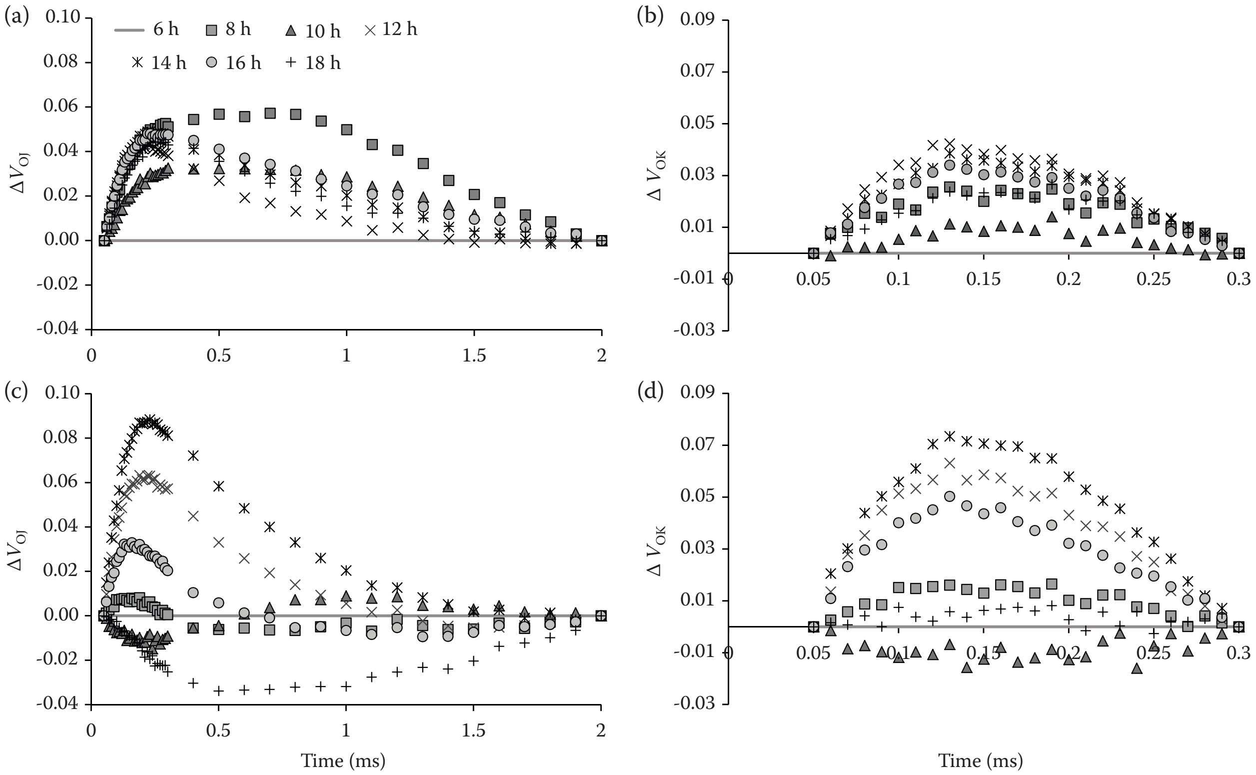

Fig. 1. Chlorophyll *a* fluorescence transient normalized between O and K ($\Delta V_{OK} = V_{OK\ (water\ deficit)} - V_{OK\ (irrigated\ plants)}$) and between O and J ($\Delta V_{OJ} = V_{OJ\ (water\ deficit)} - V_{OJ\ (irrigated\ plants)}$), evidenced as K- (a, c) and L-band (b, d), respectively, on dark-adapted leaves of Jacarandá-da-Bahia seedlings submitted to water deficit during 7 days. Measurements were performed every two hours along the day ($n = 10$)

and maximum fluorescence (F_m or F_p) were recorded and analysed according to the JIP-test (Strasser et al. 2004; Stirbet, Govindjee 2011). The relative fluorescence between points O and K (50 and 300 µs, respectively) – V_{OK} and between points O and J (50 µs and 2 ms, respectively) – V_{OJ} was calculated according to Eqs 2 (Figs 1a, b) and 3 (Figs 1c, d):

$$V_{OK} = \frac{F_t - F_0}{F_K - F_0} \tag{2}$$

where:

F_t – fluorescence at time *t* after onset of actinic illumination.

$$V_{OJ} = \frac{F_t - F_0}{F_J - F_0} \tag{3}$$

It was normalized and presented as the kinetic difference (Eqs 4 and 5):

$$\Delta V_{OK} = V_{OK(\text{treatment})} - V_{OK(\text{control})} \tag{4}$$

$$\Delta V_{OJ} = V_{OJ(\text{treatment})} - V_{OJ(\text{control})} \tag{5}$$

The kinetic difference ΔV_{OK} and ΔV_{OJ} become visible in the L- and K-bands, respectively, with peaks around 0.15 and 0.3 ms, respectively.

Statistical analysis. A completely randomised design was utilised, with 5 replicates for RWC, soil moisture and PAR and ten replicates for chlorophyll *a* fluorescence measurements. Microsoft Excel (Version 2013) was used to create graphs for the OJIP transients. RWC, soil moisture, PAR, temperature and JIP-test parameters were evaluated through variance analysis (ANOVA) and the means compared by Tukey's test at 5% probability.

RESULTS

RWC decreased ($P \leq 0.05$) along the day in T1. RWC was significantly lower ($P \leq 0.05$) in T2 at all times evaluated (Fig. 2a). Soil moisture decreased about 39% along the day in T1, with significantly lower values ($P \leq 0.05$) at 2 and 6 p.m. Soil moisture was significantly higher in T1 ($P \leq 0.05$) compared with T2 (Fig. 2a). The variation observed in soil moisture along the day in T2 was not significant ($P > 0.05$). In both treatments, the reduction of soil moisture occurred mainly between 10 a.m. and 2 p.m., during which the highest air temperatures and high PAR were recorded (Fig. 2b). Visual symptoms of leaf wilt and leaf fall were observed after 7 days in plants submitted to water stress.

Chlorophyll *a* fluorescence curves showed a typical polyphasic OJIP at all times, with J and I-steps

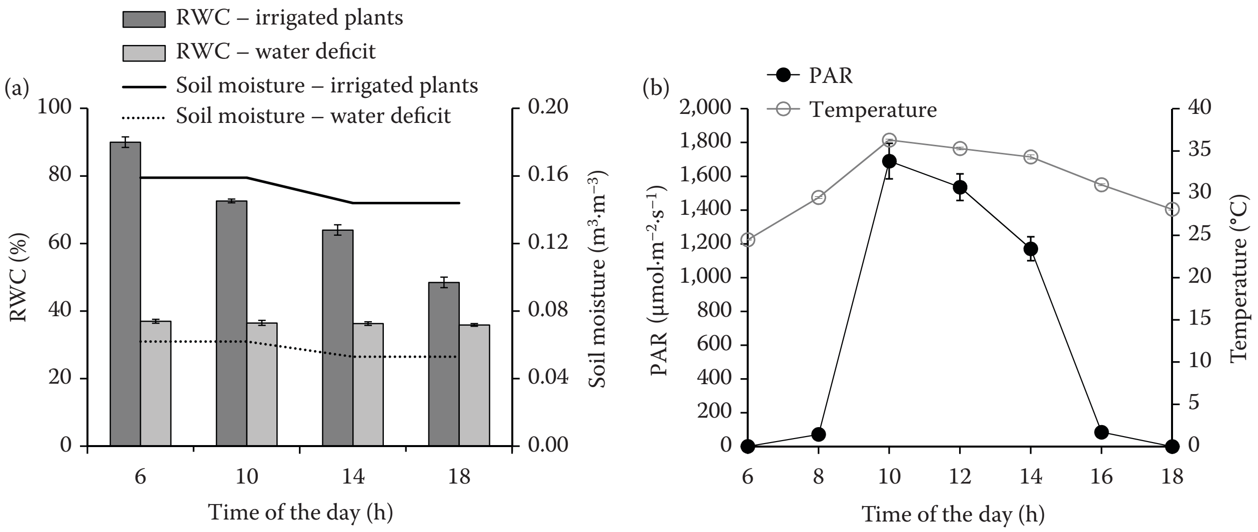

Fig. 2. Leaf relative water content (RWC) and soil moisture (a), photosynthetically active radiation (PAR) and temperature (b) along of the day, means ± standard error (n = 5)

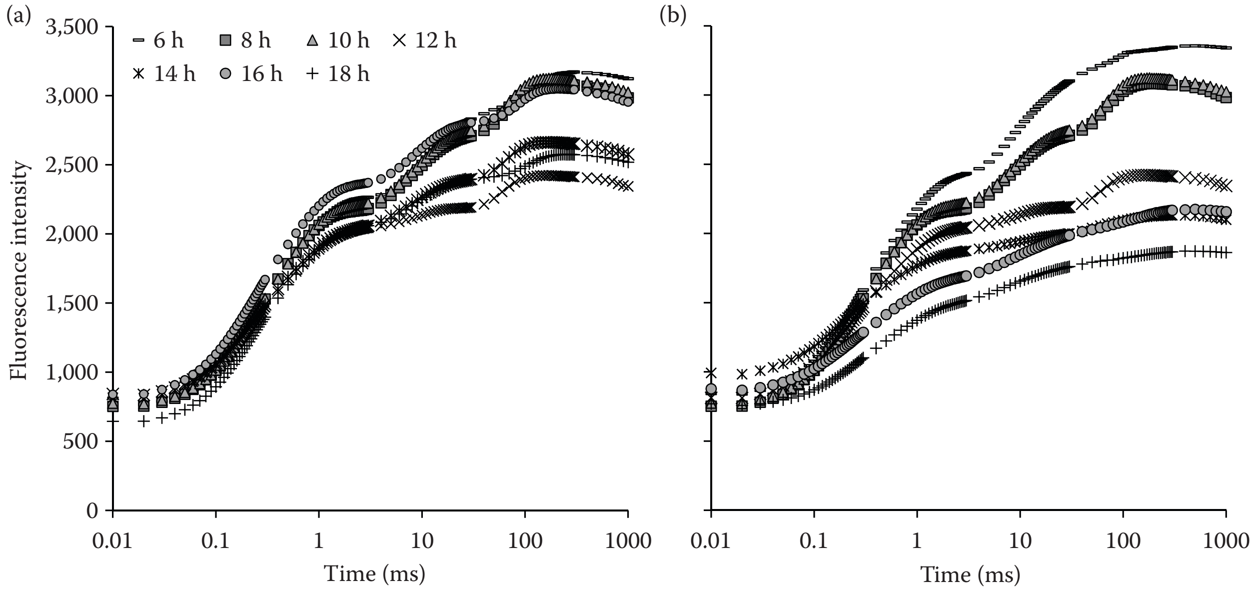

Fig. 3. Chlorophyll *a* fluorescence transient intensity in dark-adapted leave of Jacarandá-da-Bahia seedlings submitted to daily irrigation (a) and to water deficit during 7 days (b). Measures were taken every two hours along of the day (n = 10)

well defined (Figs 3a, b). However, along of the day, reductions in J, I and P-steps were observed in both treatments, being more evident in higher PAR and temperature (between 12 and 16 p.m.). Differences of the initial fluorescence (F_0) production were not observed between treatments whereas reductions of maximum fluorescence (F_m or F_p) were remarkable in T2, especially after 12 p.m. Reductions in F_m at these same times were also observed in T1, but to a lesser extent. The area above the curve between F_0 and F_m was also smaller in T2 (Figs 3a, b).

Water deficit enhanced the effects of both high temperature and irradiation on the energy connectivity of PSII subunits and OEC stability. This result can be supported by the high and positive L-band and K-band values at 12, 14 and 16 p.m. in T2, with total recovery (negative values) of the L-band curves at 18 p.m. (Fig. 1b). Under water deficit (T2), changes of K-band were more pronounced, compared to L-band (Fig. 1d). At 16 p.m., the amplitude of the K-band remained very positive compared to the L-band (Figs 1b, d).

DISCUSSION

This study was carried out to elucidate some of the photochemical mechanisms involved in the physiological responses of Brazilian rosewood seedlings submitted to water deficit for seven days.

Studies of this nature allow us to identify the action sites of the stress on the photosynthetic electron transport chain. Although significant decrease ($P \leq 0.05$) of RWC along the day was observed in watered plants, it is important to note that at 6 p.m., the RWC values were very close among treatments, evidencing that the effects of water deprivation during 7 days on the RWC values were very similar to the water deficiency from a single day.

On the other hand, the invariability of the RWC values along of the day recorded in T2 may be attributed to the low stomatal conductance values, which remained close to 10 $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ along the day (data not shown). Similar values of stomatal conductance were also obtained by Oliveira et al. (2005) and Vellini et al. (2008) in plantlets of bean (*Phaseolus vulgaris* Linnaeus) and in clones of *Eucalyptus* spp. under different water regimes in greenhouse conditions. The leaf relative water content, which reflects the water amount of the plant tissue in relation to the maximum amount of water retained in a certain moment, regulates numerous physiological processes in plant tissues, especially the activity of the photosynthetic apparatus (Lawson, Blatt 2014).

The typical increase of the OJIP curves observed in all treatments (irrigated and non-irrigated plants) indicates that all samples remained photosynthetically active (Mehta et al. 2010; Yusuf et al. 2010). Differences of the initial fluorescence (F_0) production were not observed among the treatments (Figs 3a, b). Reductions in F_m were observed in plants watered daily, although with lower amplitude. The area above the curve between the minimum (F_0) and maximum (F_m) fluorescence was also lower under water deprivation. This parameter (area) is an indicator of the electron acceptor-pool able to transfer energy from the electron transporters, including Q_A, Q_B and plastoquinones pool (Strasser et al. 1995). Reductions of area in Brazilian rosewood seedlings submitted to water deficit indicate imbalances between the electron flow from the donor side to the reaction centre and also to the acceptor side (Q_A) of PSII (Strasser 1997). The effects of salinity and freezing in plants of the genus *Crataegus* Linnaeus, evaluated through of OJIP curve, were similar to those obtained for Brazilian rosewood seedlings cultivated under water deficit, where reductions of the maximum fluorescence were registered in *Crataegus.* These results indicate that, overall, the response pattern of the OJIP curves is similar in plants of different species submitted to different stressful regimes (Percival, Fraser 2001).

In this study, water stress enhanced the effects of both high temperature and radiation on the energetic connectivity of the PSII subunits, evidenced by the positive L-band at 2 and 4 p.m. in those plants submitted to water deficit, with total recovery at 6 p.m. L-band is an indicator of energetic connectivity or grouping of PSII subunits (Strasser, Stirbet 1998). According to Yusuf et al. (2010), the L-band can assume either positive or negative values, considering the differences in energetic connectivity between treatment and control. When positive, lower connectivity is observed between PSII units (Strasser, Stirbet 1998). On the other hand, higher connectivity reflects the efficient use of the excitation energy and higher stability of the photosynthetic system (Strasser et al. 2004).

The evaluation of the K-band values evidenced higher sensitivity of the OEC to water deficiency (Figs 1c, d). Under water deficit, the K-band alterations were enhanced when compared to L-band values along of the entire day. Hence, seedling submitted to 7 days of water deprivation had higher OEC inhibition than those watered daily. Seedlings irrigated daily had positives K-band only on high PAR, while in the early morning (6 a.m.) and in the late afternoon (6 p.m.) no positive K-band was registered, suggesting total recovery of the electron donor side of PSII until the end of the day as well as during the night.

Oukarroum et al. (2009) have been suggesting that the visualization of L- and K-bands may be an excellent potential indicator to physiological disturbances in plants. Evaluating the effects of drought and re-watering in ten barley varieties (*H. vulgare*), Oukarroum et al. (2007) registered the occurrence of L- and K-bands during the phase of drought stress in all barley varieties, which were related to intolerance to drought stress. Decreased energetic connectivity between units of the PSII (L-band) was observed in wheat leaves (*Triticum aestivum* Linnaeus) cultivated under salt stress (Mehta et al. 2010). Heerden et al. (2007) analysed the modulation of photosynthesis in desert species (*Augea capensis* Thunberg and *Zygophyllum prismatocarpum* E. Meyer ex Sonder). The authors also encountered K- and L-bands appearing on the transient fluorescence for both species.

The results obtained in this study allow us stating that the water deficit caused a significant decrease in F_m and in the area below the fluorescence curve in seedlings of Brazilian rosewood. In addition, water deficit intensified the effects of high temperature and radiation on the energetic connectivity of the PSII subunits and stability of OEC, evidenced by the positive values of L- and K-bands on the times of higher incidence. Finally, the effects of water deficit were more evident on the OEC. Thus, we can conclude that the water deficit affected the photosynthetic parameters in *D. nigra*, evidencing the importance of daily irrigation to attenuate the effects of both high temperature and radiation on the photochemical activity in Brazilian rosewood seedlings, increasing their chances of effective transplant into degraded areas destined to restoration.

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