Ecophysiology of *Pilocarpus microphyllus* in response to temperature, water availability and vapour pressure deficit

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Abstract

Jaborandi is a natural source of alkaloids used in the treatment of important diseases and is therefore relevant to the pharmaceutical industry. However, the lack of information on the ecophysiological responses of jaborandi under different climatic conditions is the main limitation to its expansion in Brazil. Therefore, we investigated the effects of different environmental conditions on the ecophysiology of jaborandi seedlings, combining various ranges of mean air temperature (T), vapor pressure deficit (VPD), and water availability. For this purpose, jaborandi seedlings were grown in four different environments: cool and humid (Temp: 21.1 °C, VPD: 0.31 kPa), warm and humid (Temp: 26.8 °C, VPD: 0.34 kPa), warm and dry (Temp: 26.3 °C, VPD: 1.09 kPa), and cool and dry (Temp: 20.8 °C, VPD: 0.84 kPa). All environments presented two levels of water availability: well-irrigated (control: C) and under water stress (45% of the substrate's maximum water-holding capacity). Growth and fluorescence parameters, the crop's water stress index, and enzymatic antioxidant activity were evaluated. Our results revealed that seedlings showed reduced growth under water restriction in all treatments, resulting in lower total dry matter production, primarily due to a reduction in root system development. In the well-irrigated treatments, jaborandi seedlings exhibited greater growth when grown in warmer environments, regardless of the DPV (potential water availability). Under low-temperature conditions, a reduction in the maximum quantum yield efficiency of PSII was observed, indicating damage to photosystem II; furthermore, minimum fluorescence and enzymatic antioxidant activity increased. The greatest accumulation of dry mass was obtained when the seedlings were

subjected to high temperatures, indicating that regions experiencing these conditions are the most suitable for jaborandi cultivation.

Keywords: Amazon forest, Jaborandi, Environments, Oxidative stress, Thermal and water stress

Introduction

Deforestation in the Amazon rainforest promotes changes in the forest microclimate (Alves et al., 1999), exposing native species to adverse climatic conditions and, consequently, increasing their risk of extinction. In the period between 2017 and 2018, for example, deforestation reached an average of 7,500 km² per year (INPE, 2018). One of the main threatened native species in the Amazon rainforest is the medicinal species *Pilocarpus microphyllus*, known as jaborandi (Ibama, 2008; Rocha et al., 2014). Jaborandi is the only natural source of pilocarpine and one of the main sources of epiisopiloturin (Cncflora 2012), used in the treatment of diseases such as glaucoma, xerostomia (Agban et al. 2016; Gil-Montoya 2016), schistosomiasis (Véras et al. 2012), and others (Silva et al. 2013). Currently, jaborandi is found in the Amazon rainforest, where it is mainly exploited through extractivism (i.e., the process of extracting natural jaborandi resources), and is cultivated in the states of Piauí and Maranhão (Lima et al. 2017). In 2018, pilocarpine export revenues in Brazil exceeded US$5.5 million (Abiquifi, 2019), sparking interest among farmers, companies, the government, and research institutions, which are currently exploring ways to expand jaborandi cultivation to other Brazilian regions. Similarly, Caldeira et al. (2017) mention the possibility of expanding cultivation to other areas as an important alternative to minimize the risk of jaborandi extinction and generate additional income for family farms.

The main challenges to expanding jaborandi cultivation are the following: (i) expansion into new areas, especially in non-traditional growing regions, exposes jaborandi plants to challenging weather conditions; (ii) in some years, due to climatic variability, extreme weather events such as dry spells, excessive rainfall (which also affect the VPD), extreme high- and low-temperature events have

53 occurred (Gelcer et al. 2013; Nóia Junior and Sentelhas 2019); and (iii) with climate change, an increase
54 in temperature and evaporative demand and higher frequencies of extreme weather events, are
55 expected in both traditional and non-traditional areas (Stott 2016). All these above-mentioned points
56 are problems related to plantation production and profitability and increase the chances of extinction
57 of the species (Caldeira et al. 2017). Thus, the future of jaborandi cultivation depends on understanding
58 how jaborandi responds to different temperature ranges, water availability and vapour pressure deficit
59 (VPD).
60 Exposure of most plants to high temperatures, for example, increases respiration and
61 photorespiration, reducing photosynthetic efficiency (Yamori et al. 2014; Nóia Junior et al. 2018a), and
62 induces leaf abscission (Hikosaka et al. 2006; Taiz and Zeiger 2013). On the other hand, low
63 temperatures reduce the photosynthetic rate due to decreased stomatal conductance, resulting in
64 diffusive and metabolic limitations (Santos et al. 2011; Nóia Júnior et al. 2018b). In addition, many
65 authors have shown that plant responses to temperature can also be influenced by other climatic
66 variables, such as VPD and water availability (Marenco et al. 2014; Chaves et al. 2009; Guo et al. 2010;
67 Nóia Junior et al. 2019). However, this type of study is still lacking for jaborandi.
68 Against this background, we consider that understanding the physiological responses of jaborandi to
69 different environments will provide support for its cultivation expansion into new areas and
70 consequently its conservation. Thus, the objective of this study was to evaluate the effects of a
71 combination of different temperatures, VPD and water availability levels on the growth, crop water
72 index, chlorophyll fluorescence parameters and enzymatic antioxidants of jaborandi seedlings.
73

74 ## Material and methods

75 ### Study site and experimental design

76 The study was conducted in commercial greenhouses (model Van der Hoeven®) with controlled
77 temperature and relative humidity at the Laboratory of Meteorology and Forest Ecophysiology at the
78 Federal University of Espírito Santo, in the municipality of Jerônimo Monteiro, Espírito Santo State,

Brazil. *Pilocarpus microphyllus* seedlings were obtained from the Brazilian Agricultural Research Corporation Eastern Amazon (EMBRAPA), located in the municipality of Belém, Pará State, Brazil. The seedlings were planted in 9.5-L pots filled with a substrate composed of biostabilised pine bark, vermiculite, charcoal mill and phenolic foam. Fertilisation was carried out by adding 4.74 g L^{-1} of controlled-release fertiliser with the following nutrients: nitrogen (16%), phosphorus (8%), potassium (12%), magnesium (2%), Sulphur (5%), iron (0.4%), boron (0.02%), zinc (0.02%), copper (0.05%), manganese (0.06%) and molybdenum (0.015%).

The seedlings were 6 months old at planting, with an average height of 7.3 cm and a stem diameter of 1.4 mm. They were maintained for 15 days under conditions similar to the species' natural environment (WH) and then randomly distributed into the different experimental environments (CH, WH, WD, and CD; the meaning of these abbreviations is explained below in this subsection). Jaborandi grows naturally in the Amazon rainforest in a tropical monsoon climate (Am, warm with a short dry season), according to the Köppen climate classification (Alvares et al., 2013). The four different treatments were defined by a combination of various temperature and VPD conditions, characterized as follows: CH, low air temperature (21 °C; 'cold', C) and low VPD (0.31 kPa; 'humid', H); WH, high air temperature (26.8 °C; 'warm', W) and low VPD (0.34 kPa); WD, high air temperature (26.3 °C; 'warm', W) and high VPD (1.09 kPa; dry, D); and CD, low air temperature (20.8 °C) and high VPD, (0.84 kPa). These four environments represent the majority of climatic conditions found in Brazil (Alvares et al. 2013). Each of these four environments occurred in different greenhouses. Also, there were two water availability levels within each of the above-mentioned treatment: well-watered (control: C) and water-stressed (45% of the maximum substrate field capacity; further information is presented in the Sect. 2.3). The experiment followed a completely randomized design with four replicates; each replicate was represented by one plant. The experiment period was from July 2017 to April 2018, i.e. the jaborandi seedlings were exposed to the above-mentioned experimental conditions for 270 days. The maximum, minimum and average photoperiod during the period were, respectively, 13.24, 10.78 and 12.2 h.

105 **Microclimatic characterization**

106 An automatic weather station was installed for microclimatic characterization of the environment
107 within each of the four greenhouses. This station consisted of a CS500 temperature and relative
108 humidity sensor (Campbell Scientific, Inc., Logan, UT, USA). Data were stored on a CR-10x data logger
109 (Campbell Scientific, Inc., Logan, UT, USA), with scans every 10 seconds and average values stored at
110 5-minute intervals. Vapor pressure deficit (VPD) values were calculated as the difference between
111 saturation pressure and partial pressure of water vapor. Microclimatic characterization was performed
112 over the 270 days of the experimental period. Daily temperature, VPD, and relative humidity in the
113 four studied environments, along with their average values, are presented in Figure 1 and Table 1,
114 respectively.

115 The air temperature of the greenhouses was controlled by an evaporative cooling system (pad cooling),
116 air conditioners and heaters which were activated by temperature controllers (Full Gauge ®, MT-543Ri
117 plus). Relative humidity values were maintained by using humidity controllers (Full Gauge ®, AHC-80
118 plus) which are based on psychrometry, and a fogger nozzle fogging system. The variations in
119 temperature and relative humidity values of the systems were done by Sistrad ® software. This
120 software checked and changed (when necessary) the greenhouse temperature and relative humidity
121 every 30 min during the day and every hour during the night to simulate the daily environmental
122 conditions in each greenhouse in the study (Fig. 1).

123

124 **Monitoring of water availability levels**

125 Irrigation was carried out based on daily weighing of the pots with seedlings, replacing the water lost
126 by evapotranspiration (Freire et al. 1980). For the well-watered treatment, plants were irrigated daily,
127 and for the water-stress treatment (*D*), irrigation was applied when soil moisture reached 45%
128 (measured gravimetrically) to achieve the maximum substrate field capacity (this procedure was
129 repeated throughout the entire experimental period).

130

Plant growth analysis

After the experimental period, the dry mass (DM) for each organ was obtained after drying leaves, stems and roots at 65 °C until constant weight (The precision of the equipment used to determine the dry mass was 0.0001 g). Heights were measured with a ruler (0.1 cm precision), diameters with a digital calliper (0.01 mm precision) and leaf area (LA) with the leaf area integrator model LI-3100 (Li-Cor Inc, Lincoln, Nebraska, USA).

Crop water index

The crop water stress index (CWSI) was calculated according to the equation proposed by Idso (1982):

$$CWSI = \frac{(T_{dry} - T_{leaf})}{(T_{dry} - T_{wet})}$$

where *T*leaf is the mean leaf temperature exposed to the different treatments, *T*wet is the mean leaf temperature of a leaf saturated with water (saturation was performed with water spray 5 min prior to the leaf temperature measurement) and *T*dry is the leaf temperature under non-transpiring conditions, coated with Vaseline for stomatal closure. Values of CWSI close to 1 indicate that plants are stressed, and close to 0, that plants are not stressed.

Thermal images were acquired at 10:00 AM and 12:00 PM using a FLIR T430sc thermal imaging camera (FLIR Systems, Wilsonville, OR, USA), with a resolution of 320 × 240 pixels. Analysis was performed at 10:00 AM because this is the time of highest photosynthetic activity in the plants, and at 12:00 PM because this is the time of greatest solar radiation availability. The distance between the camera and the plants was 0.4 m, with an emissivity of 0.96. Four thermal images were taken per treatment (images were taken from four different plants, each plant considered a replicate), and ten temperature samples were taken from each thermal image (ten samples from the same leaf). The images were processed using FLIR Tools software, measuring ten samples from each leaf to obtain the mean leaf temperature for the respective treatments. Measurements were taken only once, at the end of the experimental period.

Fluorescence parameters

We analysed the following chlorophyll fluorescence parameters: the maximum quantum efficiency of PSII (Fv/Fm), minimum fluorescence (Fo), using a FluorPen model FP 100 (Photon Systems Instruments, Brno, Czech Republic). Measurements were performed on fully expanded upper middle leaves, adapted to the dark for 30 min, with a light saturation pulse of 1.500 µmol m^{-2} s^{-1}, as suggested by Baker (2008). Data were collected at 4 am (pre-dawn) and at 12 pm (highest incidence of solar radiation), because they are indicated to determine the damage to the photochemical phase of photosynthesis based on Murchie and Lawson (2013). Four replications were collected per treatment: one replication consisting of measurements made on three different leaves per plant (i.e. three samples per plant). The distance between the leaves and the equipment was the same in all measurements, being around 1 mm. All these parameters (CWSI, Fv/Fm and Fo) were measured in the last week of the experimental period.

Enzymatic antioxidants

For enzymatic antioxidants analysis, fully expanded leaves were taken from the upper third of the plant, at the end of the experimental period. The enzyme extracts were obtained following the methodology proposed by Peixoto et al. (1999). Briefly, 0.3 g of the leaf sample was macerated in liquid nitrogen, and 2 mL of a solution composed of 0.1 M concentration potassium phosphate buffer (pH 6.8), ethylenediaminetetraacetic acid (0.1 mM), phenylmethylsufonic fluoride (1 mM) and polyvinylpolypyrrolidone (1%) was added.

Catalase activity (CAT, EC 1.11.1.6.) was determined according to the methodology of Havir and Mchale (1987). The reaction mixture (2.9 mL) consisted of 50 mM potassium phosphate buffer (pH 7.0) and 12.5 mM hydrogen peroxide, and the reaction was started by adding 100 µL of the enzyme extract. The reaction of the extract was evaluated twice in a UV–visible spectrophotometer model Multiskan Go (Thermo Scientific, Multiskan GO, Finland) at 25 °C in absorbance at a wavelength 240 nm, in the

initial seconds and in the first minute of the reaction. The CAT activity was quantified based on the hydrogen peroxide molar extinction coefficient (36 mM^{-1} cm^{-1}), according to Anderson et al. (1995). Ascorbate peroxidase activity (APX, EC 1.11.1.11) was determined according to the methodology proposed by Nakano and Asada (1981) and adapted by Koshiba (1993). The reaction mixture for APX (2.9 mL) was composed of 50 nM potassium phosphate buffer (pH 6.0), 0.8 mM ascorbic acid and 1 mM hydrogen peroxide. The reaction started with the addition of a 100-µL aliquot of the enzyme extract; the reading was performed by a spectrophotometer at 290 nm and 25 °C at the beginning and after 1 min of the reaction. The APX activity was determined based on the molar extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Superoxide dismutase activity (SOD, EC 1.15.1.1) was determined according to the methodology proposed by Del Longo et al. (1993), with a reaction mixture (2.97 mL) composed of 50 nM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM p-nitro tetrazolium blue, 0.1 mM ethylenediaminetetraacetic acid and 2 µM riboflavin. The reaction was started with the addition of 30 µL of the crude enzyme extract. Subsequently, the 560-nm absorbance spectrophotometer was read and conferred on the activity of the blue formazan compound produced by the *p*-nitro tetrazolium blue photoreduction reaction (Giannopolitis and Ries 1977). The SOD unit activity was defined as the enzyme content capable of inhibiting 50% of the *p*-nitro tetrazolium blue photoreduction reaction (Beauchamp and Fridovich 1971). The measurements were performed at the end of the experimental period.

Statistical analysis

Statistical analyses were performed using R software (R Core Team 2017). All data were subjected to a pooled analysis of variance (ANOVA) (considering the fixed effects: environments and water availability levels) (Barros and Dias 2009), and means were compared using Tukey's test ($p \leq 0.05$) to assess the interaction between environments and water availability levels. The normality and homogeneity of the data ($p \leq 0.05$) were analyzed using the Shapiro-Wilk test (1965) and Hartley's F-

test (1950). Pooled analysis was performed due to the lack of replication of each environment. Interaction graphs between environments (WD, WH, CD, CH) and water availability level treatments were plotted when significant. When the interaction was not significant, the environments (WD, WH, CD, CH) were compared by grouping both irrigation treatments and we present all the results of the ANOVA on the supplementary material (Tables S1, S2, S3 and S4).

Results

Seedling growth

Seedlings exposed to high temperatures (WH and WD) showed greater total dry mass accumulation under well-irrigated conditions ($p < 0.001$, Fig. 2a). The results also show that different VPD values applied under well-irrigated conditions did not affect total dry mass accumulation within the same temperature range, although they did affect root dry mass. However, jaborandi seedlings exhibited greater root growth in warm environments with high VPD levels (WD) under water stress conditions. Seedlings exposed to the CD treatment showed a 73% reduction in total dry mass accumulation compared to the WD treatment.

Jaborandi seedlings had higher shoot dry mass in the WH and WD than other treatments (Fig. 2b). Shoot dry mass was on average 57% higher in WH and WD than in CD. In all environments, shoot dry mass was significantly reduced by the water-stressed conditions ($p < 0.001$). A combined condition of low temperature and high humidity (CH) limited root mass dry matter growth ($p < 0.001$, Fig. 2c). However, there were no differences in root growth between the environments when the seedlings grew under water-stressed conditions ($p < 0.001$).

The different environments and water availability levels significantly affected the height growth and leaf area of the jaborandi ($p \leq 0.001$, Fig. 3); there was no interaction between environments and water availability for height and leaf area, but both of them individually affected these growth parameters (Table S1 in the supplementary material). High temperatures (WH and WD) positively influenced height growth (Fig. 3a). The seedlings grown in the WH and WD had a 44% increase in the height compared

234 to seedlings exposed to CH and CD ($p \leq 0.001$, Fig. 3). The WH increased the leaf area (Fig. 3c). The
235 well-watered jaborandi seedlings had higher growth than the water-stressed seedlings, with 45 and
236 51% greater height and leaf area, respectively.
237 Stem diameter increased significantly in response to high temperatures in the WH (+36%) and WD
238 (+31%) treatments, compared to the CH and CD treatments. Water stress reduced diameter growth in
239 all environments studied ($p < 0.001$). However, water limitation had a greater negative impact in the
240 warm environment with low DPV (WH).
241
242 **Crop water stress index (CWSI)**
243 The effects of different environments and water availability levels on jaborandi seedlings can be
244 expressed via the CWSI (Fig. 4). The CWSI values were higher at 12 pm, (Fig. 4b), being that time more
245 stressful than 10 am. The CWSI was statistically different among the studied environments ($p < 0.001$,
246 Table S2 in the supplementary material). The highest CWSI values for both water availability levels
247 were obtained in high-VPD environments (WD and CD at 12 pm). For seedlings grown in WD and CD
248 (12 pm), for example, CWSI values were 22 and 26% higher than those of WH for well-watered and
249 water-stressed seedlings, respectively. CWSI in response to water availability levels had a significant
250 effect ($p < 0.001$) on jaborandi seedlings at 10 am and 12 pm (Fig. 4). Within each environment, there
251 was a significant increase in CWSI for seedlings that grew under water-stressed condition. For water-
252 stressed seedlings, the environments characterised as cold (CH and CD) had the most stressful effect
253 (i.e. higher CWSI values). Similar results were found in WD at 12 pm.
254
255 **Chlorophyll fluorescence parameters**
256 On the one hand, chlorophyll a fluorescence parameter, presented as maximum quantum efficiency
257 of PSII (Fv/Fm) was influenced by the treatments tested ($p < 0.05$, Fig. 5). The results were similar for
258 all environments when seedlings grew under well-watered conditions (Fig. 5a). On the other hand, in

water-stressed conditions at 4 am, seedlings exposed to low temperatures and high VPD (CD) had Fv/Fm value of 0.7, which might indicate damage to the photosystem II.

At noon, Fv/Fm showed similar results in high-temperature environments (WH and WD) (Fig. 5b). In these environments, the value of Fv/Fm was 23% higher than those obtained in the cold environment and with high VPD (CD). The Fv/Fm was also negatively impacted by water deficit, and its value reduced from 0.61 in well-watered seedlings to 0.56 in water-stressed treatment (Table S3 in the supplementary material).

Regarding the minimum fluorescence (Fo) results at 4 am, the greatest limitation in fluorescence recovery efficiency was observed in the CD treatment ($p < 0.001$, Fig. 6a). At midday, seedlings exposed to dry environments (WD and CD) showed lower fluorescence recovery efficiency (Fig. 6b), indicating the effect of high VPD on seedling physiological processes. Simultaneously, seedlings exposed to the WD environment exhibited the lowest fluorescence recovery efficiency. Furthermore, water-stressed seedlings showed Fo values 12% lower than those of well-watered seedlings (Table S3 in the supplementary material), indicating that Fo was also affected by water limitation.

Activity of enzymatic antioxidants

Our results reveal that the production of the enzymes catalase (CAT) and ascorbate peroxidase (APX) was affected by the environment and by water availability ($p < 0.001$, Fig. 7a and b). Comparing the studied environments, CAT and APX had the lowest activity in WH for well-watered and water-stressed seedlings.

Within each environment, water limitation increased the enzymatic activity of CAT and APX. The highest CAT activity (Fig. 7a) occurred in the environment characterized as cold and with low VPD (CH). By contrast, seedlings exposed to the environment characterized as warm and with high VPD (WD) had the highest APX activity (Fig. 7b). In addition, the effect of the water limitation was more intense in the WH environment, since the CAT and APX activities in water-stressed seedlings were 80% greater than in well-watered seedlings.

285 The production of the antioxidant enzyme superoxide dismutase (SOD) differed statistically among the
286 different environments ($p < 0.001$, Fig. 7c). The production of this enzyme was lower in the warm
287 environment and under low VPD (WH). Water stress significantly increased SOD activity by 22% from
288 well-watered treatment ($p < 0.002$, Table S4 in the supplementary material).
289

290 **Discussion**

291 This study showed how temperature, VPD and water availability can influence the growth of jaborandi
292 seedlings. This indicates the challenge that Brazilian producers and researchers face in the process of
293 expanding this species to environments with adverse climatic conditions, as well as in actions to reduce
294 the risk of extinction of this species.
295 Seedling growth and biomass production in WH and WD increased with increasing temperature.
296 Similar results have been reported by Ribeiro et al. (2017) for Amazonian species, Xavier et al. (2017)
297 for Atlantic Forest species, and Xavier et al. (2018) for eucalyptus. At lower temperatures (CH and CD),
298 seedling growth (shoot and root) and leaf expansion were negatively affected. The negative effects of
299 lower temperatures on Amazonian species have also been observed by other authors (Ribeiro et al.
300 2017; Coelho et al. 2013; Nóia Junior et al. 2018b).
301 Our results indicate that low temperature had a negative effect on maximum quantum yield efficiency
302 of PSII (Fv/Fm) of jaborandi species from CH and CD, especially at noon. Low Fv/Fm values are related
303 to photoinhibition, i.e. light-induced reduction of photosynthetic capacity. The low Fv/Fm values
304 usually corresponded with low stomatal conductance (Prieto et al. 2009). According to Nóia Júnior et
305 al. (2018a), low temperature reduces stomatal conductance, and the carboxylation process of rubber
306 trees, a native to the Amazon Rainforest, such as jaborandi. The stomatal closure may generate a
307 reduction of the CO2 available within the stomatal cavity, affecting the carboxylation process and the
308 momentary photosynthetic potential of the species; this reduction can generate a dynamic
309 photoinhibition (reversible reductions of Fv/Fm) (Prieto et al. 2009).

Jaborandi seedlings were also sensitive to different VPD values. Environments with higher VPD (WD and CD) promoted an increase in the CWSI index (Fig. 4). This index ranges from 0 to 1, where 0 represents a well-hydrated condition and 1 a totally stressful one (Jackson et al. 1981; Vazquez 2013). The indicative stress threshold is 0.7 (Matese et al. 2018; Bellvert et al. 2014), but may vary among species. Bellvert et al. (2014), for example, report vine CWSI values between 0.3 and 0.5 as moderately stressed and equal to or above 0.7 as severe stress. According to García-Tejero et al. (2016) and Pou et al. (2014), there is a strong correlation between leaf temperature and stomatal opening, which indicates that stomatal closure under conditions of high VPD and/or low water availability may have caused the increase in the CWSI. Thus, our results show, through the CWSI, that the limitation of water in the environment and substrate might have caused changes in the heat dissipation and leaf cooling processes of jaborandi seedlings, leading to an increase in leaf temperature (Fig. 4).

There were changes in quantum yield (Fv/Fm) and minimum fluorescence (Fo). According to Murchie and Lawson (2013) and to Zha et al. (2017), the Fv/Fm ranges from 0.77 to 0.81 for most plant species in healthy and unstressed conditions. Under conditions of low temperatures and high VPD (CD) at 4 am, there was a reduction of Fv/Fm in jaborandi seedlings to 0.68, indicating that throughout the night, the photosystem II efficiency of the seedlings was not recovered. At noon, after 30 min of dark adaptation, this effect was even greater, as there was a reduction in Fv/Fm to 0.54 and 0.39 in CH and CD, respectively (Fig. 5). We measured Fv/Fm after 263 days of experimentation, which gives the indication that the maximum quantum efficiency of PSII reduction would not be recovered by the seedlings in the CH and CD environments. In general, the decrease in Fv/Fm occurs as a result of PSII photochemistry inactivation and/or increased dissipation of thermal energy from PSII-associated chlorophyll antennas (Adams et al. 2013). Our results also indicate an increase in minimal fluorescence at 4 am in CD environments, and at noon (12 pm) in the CH, WD and CD environments (Fig. 6).

Water stress has an inhibitory effect on plant growth rate (Tardieu et al. 2011; Zlatev and Lidon 2012). Water restriction limited growth of jaborandi seedlings by reducing their height, diameter, leaf area and total biomass. Also, our results showed that APX, SOD and CAT enzyme activities increased, as well

as an increase in CWSI and Fo and a reduction of Fv/Fm under water stress conditions. Although the stomatal and mesophyll conductance were not measured in this study, it is important to mention that that their reduction is indicated by several authors as the main cause of the reduction of photosynthetic rates under water stress (Warren et al. 2011; Cano et al. 2014). Other processes such as the inhibition of ribulose-1,5-bisphosphate-carboxylase-oxygenase (Rubisco) activity and reduced regeneration capacity of ribulose-1,5-bisphosphate (RuBP) are also cited as important inhibitors of photosynthesis (Tezara et al. 2002; Thimmanaik et al. 2002).

The different environments studied also influenced the biochemical processes of jaborandi seedlings. The activity of the antioxidant enzymes SOD, APX, and CAT increased in response to high levels of VPD and water limitation (Fig. 7); similar results have been reported by Tang et al. (2018). Activation of the antioxidant system is an internal physiological regulation in response to environmental stimuli (Salazar-Parra et al. 2012). Several studies show that under adverse conditions, the plant is protected against the effects of ROS by increased antioxidant enzyme activity (Sánchez-Rodríguez et al. 2012; Li et al. 2017; He et al. 2014). The activity of the antioxidant enzymes SOD, APX, and CAT in the defense system, for example, is an adaptive response of the plant to drought tolerance (Aghaie et al. 2018), as can also be seen in our results.

Our results revealed that the jaborandi seedlings decreased their total dry mass when submitted to conditions with limited water availability, irrespective of the environment. The effects of the limited water availability were more prominent in the root dry mass. When submitted to a well-watered condition, jaborandi seedlings showed a higher dry mass when cultivated in warmer environments, independently of the VPD. Under low temperatures, there was a reduction in quantum yield, an increased minimum fluorescence and higher enzymatic activity, indicating damage to photosystem II, limiting physiological processes and reducing growth. To the best of our knowledge, our study is the first to show the sensitivity of jaborandi ecophysiology to different environmental conditions. Our results point out that regions with high temperatures and without water restriction (e.g. states located in Northern Brazil, northern of Mato Grosso State, and in central regions of Brazil where irrigation is

possible, such as agricultural areas around the São Francisco river) could be the most suitable ones for the growth of jaborandi seedlings in Brazil.

Author contributions statement GCA coordinated research, collected, analysed data, interpreted data and wrote the manuscript. RSNJ wrote the manuscript and aided with data interpretation. JEMP, JVT provided support, advice, and guidance throughout the experiment. JEMP, RSNJ, MFM, BSO, RACJJ and EOG contributed to the revision of the manuscript and also provided insights into different aspects of the work and aided with the statistical analysis. MDSF and TMTX assisted in laboratory experiments.

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583

Table 1. Characteristics of the greenhouse microclimate with controlled temperature and relative humidity throughout the experiment

Climatic parameters	CH	WH	WD	CD
	Air temperature (°C)			
T_{min}	15.5	21.3	20.7	15.0
T_{avg}	21.1	26.8	26.3	20.8
T_{max}	29.5	34.7	34.0	30.0
	Relative humidity (%)			
UR_{min}	70.5	70.7	39.8	42.4
UR_{avg}	88.4	91.0	65.7	65.0
UR_{max}	98.1	99.9	83.9	77.4
	Vapour pressure deficit (kPa)			
DPV_{min}	0.03	0.00	0.33	0.35
DPV_{avg}	0.31	0.34	1.09	0.84
DPV_{max}	0.97	1.27	2.49	2.00

Treatments: CH: Low temperature and high relative humidity, WH: high temperature and high relative humidity, WD: high temperature and low relative humidity and CD: low temperature and low relative Humidity.
Tavg average air temperature, *Tmin* minimum air temperature, *Tmax* maximum air temperature, *VPDavg* average vapour pressure deficit, *VPDmin* minimum vapour pressure deficit, *VPDmax* maximum vapour pressure deficit, *RHavg* average relative humidity, *RHmin* minimum relative humidity, *RHmax* maximum relative humidity.

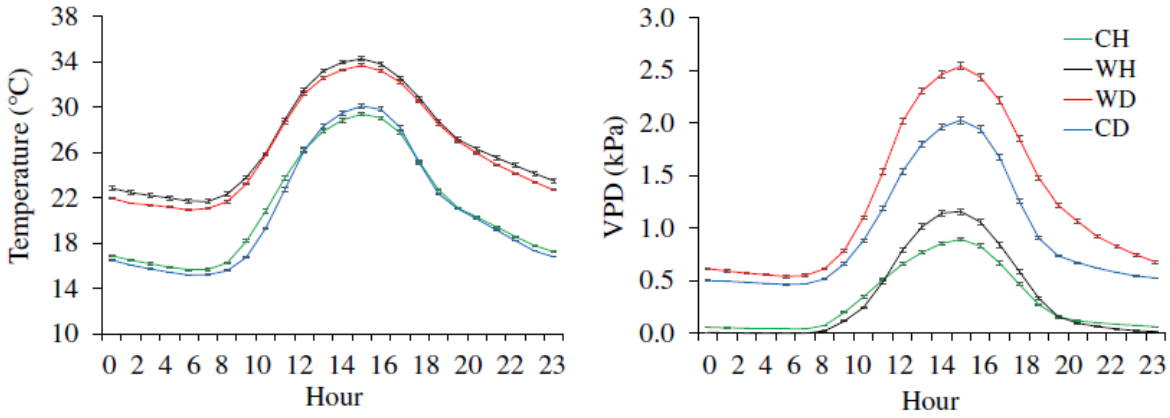

Figure 1. Daily variation in air temperature and vapour pressure deficit (VPD) during the experimental period (From July 2017 to April 2018), simulating four environmental conditions. Low temperature and low VPD (CH), high temperature and low VPD (WH), high temperature and high VPD (WD) and low temperature and high VPD (CD). Values represent mean ± standard deviation.

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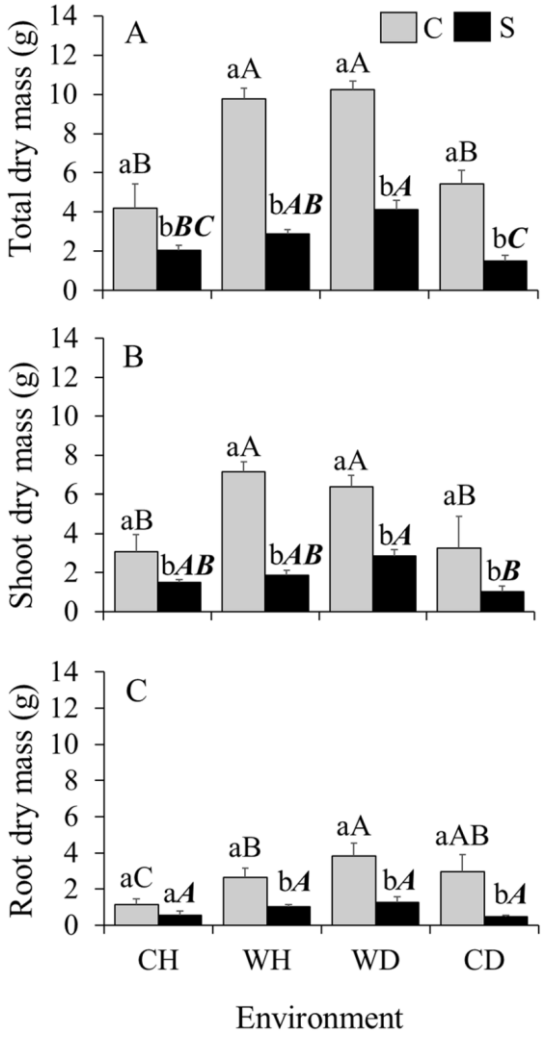
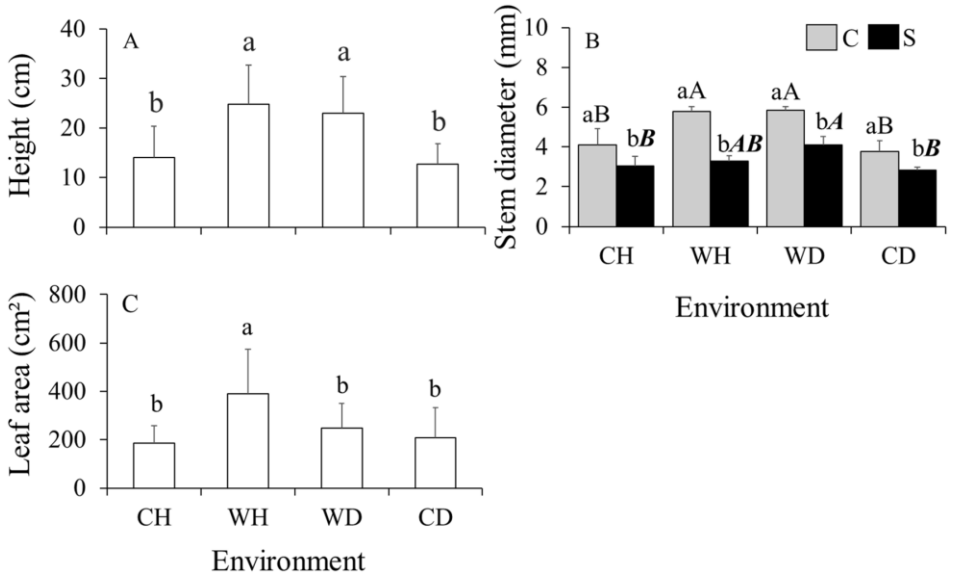


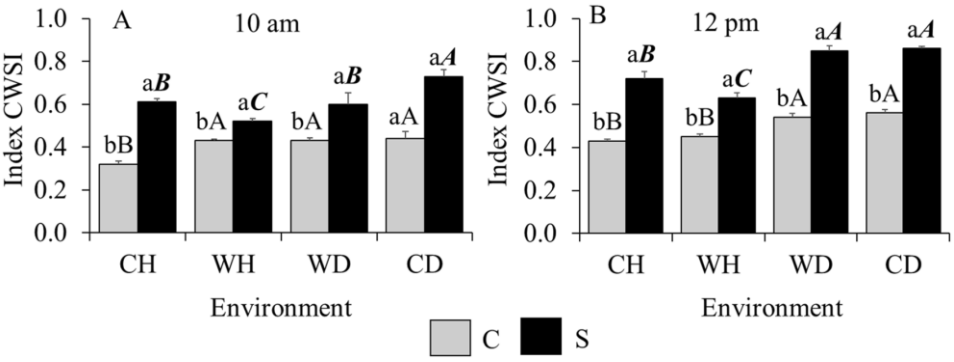
605

606 **Figure 2.** Total dry mass (**a**), aerial part dry mass (**b**) and root dry mass (**c**) of jaborandi seedlings submitted to
607 different thermal and water availability regimes. Environments: cold and humid (CH); warm and humid (WH);
608 warm and dry (WD); and cold and dry (CD). Water availability levels: well-watered (or control: C) and
609 waterstressed (S). Different letters represent statistically significant differences between treatments (Tukey, $p \leq$
610 0.05). The statistical differences between environments are represented by the uppercase letters (uppercase for
611 control and uppercase bold/italic for stressed) and between water availability levels within each environment by
612 lowercase letters. Values represent means ± standard deviation. The results from the analysis of variance
613 (ANOVA) taking into account the fixed effects of the different environments and water availability levels are
614 presented in the table S1 in the supplementary material.



615

616 **Figure 3.** Height (**a**), diameter (**b**) and leaf area (**c**) of jaborandi seedlings subjected to different temperature, VPD
617 and water availability. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold
618 and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters
619 represent statistically significant differences between treatments (Tukey, $p \leq 0.05$). For diameter (B), uppercase
620 letters compare the statistical difference by environment (upper case for control and upper case bold/italic for
621 stressed), and lowercase letters represent the statistical difference between water availability levels. Values
622 represent the mean ± standard deviation. Graphs of the interaction between environments (WD, WH, CD, CH)
623 and water availability levels were plotted when the interaction was significant (panels with gray and black bars);
624 when the interaction was not significant, environments (WD, WH, CD, CH) were compared by grouping both
625 irrigation treatments (panels with white bars). The results of the analysis of variance (ANOVA), considering the
626 fixed effects of the different environments and water availability levels, are presented in Table S1 of the
627 supplementary material.
628



629

630 **Figure 4.** Crop water stress index (CWSI) measured at 10 am (**a**) and 12 pm (**b**) in jaborandi leaves under different
631 environmental conditions. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and
632 cold and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters
633 represent statistically significant differences between treatments (Tukey, $p \leq 0.05$). The statistical differences
634 between environments are represented by the uppercase letters (uppercase for control and uppercase
635 bold/italic for stressed), and between water availability levels within each environment by lowercase letters.
636 Values represent means ± standard deviation. The results from the analysis of variance (ANOVA) taking into
637 account the fixed effects of the different environments and water availability levels are presented in the Table
638 S2 in the supplementary material.

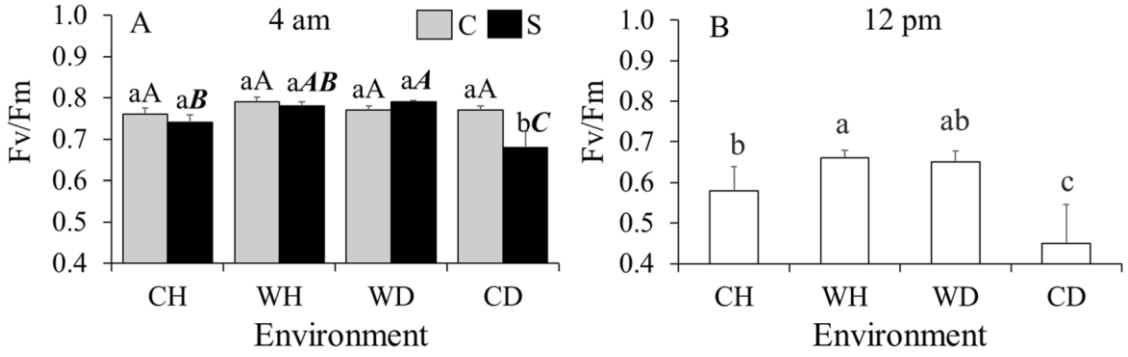


Figure 5. Quantum yield (Fv/Fm) at 4 am (**a**) and 12 pm (**b**) on jaborandi leaves under different environmental conditions. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters represent statistically significant differences between treatments (Tukey, $p \leq 0.05$). For Fv/Fm (**a**), capital letters compare the statistical difference by environment (upper case for control and bold/italic upper case for stressed), and lowercase letters represent the statistical difference between ambient water availability levels. Values represent the mean ± standard deviation. Graphs of the interaction between environments (WD, WH, CD, CH) and water availability levels were plotted when the interaction was significant (panels with gray and black bars); when the interaction was not significant, environments (WD, WH, CD, CH) were compared by grouping both irrigation treatments (panels with white bars). The results of the analysis of variance (ANOVA), considering the fixed effects of the different environments and water availability levels, are presented in Table S3 of the supplementary material.

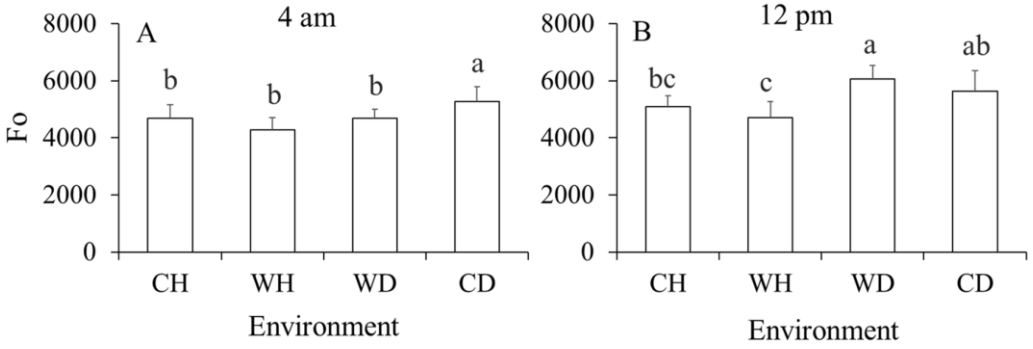


Figure 6. Minimum fluorescence (Fo) at 4 am (**a** and **b**) and at 12 pm (**c** and **d**) in jaborandi leaves under different environmental conditions. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold and dry (CD). Different letters represent statistically significant differences between treatments (Tukey, $p \leq 0.05$). The values represent the mean ± the standard deviation. The results of the analysis of variance (ANOVA), considering the fixed effects of the different environments and levels of water availability, are presented in Table S3 of the supplementary material.

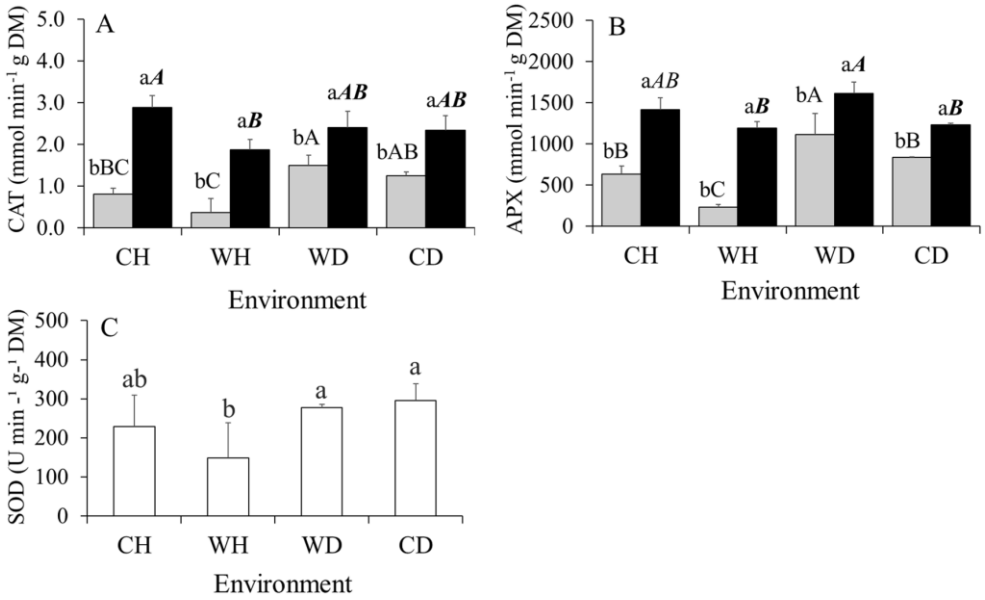


Figure 7. The activity of enzymatic antioxidants catalase (**a** CAT), ascorbate peroxidase (**b** APX) and superoxide dismutase (**c** SOD) of jaborandi leaves exposed to different environmental conditions. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters represent statistically significant differences between treatments (Tukey, $p \leq 0.05$). For CAT and APX (**a** and **b**), uppercase letters compare the statistical difference by environment (upper case for control and bold/italic case for stressed), and lowercase letters represent the statistical difference between ambient water availability levels. Values represent the mean ± standard deviation. Graphs of the interaction between environments (WD, WH, CD, CH) and water availability levels were plotted when the interaction was significant (panels with gray and black bars); when the interaction was not significant, environments (WD, WH, CD, CH) were compared by combining both irrigation treatments (panels with white bars). The results of the analysis of variance (ANOVA), considering the fixed effects of the different environments and water availability levels, are presented in Table S4 of the supplementary material. Dry matter (DM).

Supplementary material

Table S1. Growth variables of jaborandi seedlings submitted to different thermal and water availability regimes. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters represent statistically significant differences between treatments (Tukey, $p \leq 0.01$). The values are the mean values of each variable for the individual treatments.

Causes of variation	Total dry mass	Shoot dry mass	Root dry mass	Height	Diameter	Leaf area
	- - - - - - - - - - - - g - - - - - - - - - -			cm	mm	cm^2
Environment	67.56**	18.61**	15.07**	17.95**	18.28**	7.78**
Irrigation	373.50**	99.12**	102.67**	54.30**	71.40**	29.63**
Environment x irrigation	18.63**	6.61**	6.74**	1.48^{ns}	3.77**	2.39^{ns}
			Environments			
CH	3.13 b	2.27 b	0.85 c	14.00 b	3.56 b	186.27 b
WH	6.33 a	4.50 a	1.83 b	24.78 a	4.52 a	390.44 a
WD	7.17 a	4.63 a	2.54 a	22.96 a	4.97 a	247.56 b
CD	3.47 b	2.14 b	1.71 b	12.67 b	3.29 b	208.62 b
			Water avalability levels			
C	7.41 a	4.96 a	2.64 a	23.95 a	4.87 a	347.68 a
S	2.64 b	1.81 b	0.83 b	13.25 b	3.30 b	168.76 b
C.V. (%)	13.88	26.40	29.11	22.07	12.81	36.00

** = significant at the 1% probability level; ns = not significant; C.V. = coefficient of variation.

Table S2. Crop water stress index (CWSI) of jaborandi seedlings submitted to different thermal and water availability regimes. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters represent statistically significant differences between treatments (Tukey, $p \leq 0.01$). The values are the mean values of each variable for the individual treatments.

Causes of variation	CWSI	
	10 am	12 pm
Environment	23.36**	132.33**
Irrigation	365.16**	1332.51**
Environment x irrigation	19.11**	14.93**
Environments		
CH	0.46 c	0.58 b
WH	0.47 c	0.54 c
WD	0.52 b	0.70 a
CD	0.56 a	0.71 a
Water avalability levels		
C	0.40 b	0.50 b
S	0.62 a	0.76 a
CV%	6.23	3.25

** = significant at the 1% probability level; ns = not significant; C.V. = coefficient of variation.

Table S3. Quantum yield (Fv/Fm) and minimum fluorescence (F_0) at 4 am and 12 pm, respectively, of seedlings submitted to different thermal and water availability regimes. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters represent statistically significant differences between treatments (Tukey, $p \leq 0.01$). The values are the mean values of each variable for the individual treatments.

Causes of variation	F_v/F_m		F_0	
	4 am	12 pm	4 am	12 pm
Environment	12.68**	24.48**	9.20**	13.60**
Irrigation	12.85**	5.76**	20.07**	22.15**
Environment x irrigation	9.10**	2.23^{ns}	1.03^{ns}	0.37^{ns}
Environments				
CH	0.75 bc	0.58 b	4683.37 b	5094.25 bc
WH	0.79 a	0.66 a	4283.62 b	4698.50 c
WD	0.78 ab	0.65 ab	4675.62 b	6065.25 a
CD	0.73 c	0.45 c	5261.50 a	5623.62 ab
Water avalability levels				
C	0.77 a	0.61 a	4428.62 b	4988.50 b
S	0.75 b	0.56 b	5023.43 a	5752.31 a
CV%	2.77	9.47	7.94	8.55

** = significant at the 1% probability level; ns = not significant; C.V. = coefficient of variation.

Table S4. The activity of enzymatic antioxidants catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) of leaves jaborandi submitted to different thermal and water availability regimes. Environments: cold and humid (CH); warm and humid (WH); warm and dry (WD); and cold and dry (CD). Water availability levels: well-watered (or control: C) and water-stressed (S). Different letters represent statistically significant differences between treatments (Tukey, $p \leq 0.01$). The values are the mean values of each variable for the individual treatments.

Causes of variation	CAT	APX	SOD
	mmol min^{-1} g MS		cm^2
Environment	9.88**	30.16**	9.97**
Irrigation	135.31**	186.89**	7.89**
Environment x irrigation	4.68**	7.18**	0.90^{ns}
Environments			
CH	1.84 a	1024.17 b	228.82 ab
WH	1.12 b	713.89 c	149.06 b
WD	1.95 a	1365.99 a	277.56 a
CD	1.79 a	1034.78 b	296.06 a
Water avalability levels			
C	0.98 b	703.28 b	208.65 b
S	2.37 a	1366.14 a	266.99 a
CV%	23.08	13.25	24.70

** = significant at the 1% probability level; ns = not significant; C.V. = coefficient of variation.