



## **Physiological Study of Cupuaçu [*Theobroma grandiflorum* (Willd. ex. Spreng.) Schum.,] Tree Progenies Subjected to Water Deficiency**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors JGP, LCS, RLC and CFON designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RMA, DJPS and JSST participated in the management of the experiment from the implantation to the data collection. Authors AVCB, RSO and SSC was responsible for collecting, tabulating and analysing the data. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** The study aimed to investigate the physiological behaviour of cupuaçu tree progenies subjected to water deficiency.

**Study Design:** The experimental design was completely randomised, in a 2x7 factorial scheme (2 water regimes: with and without water deficiency and 7 cupuaçu tree genotypes), totalling 14 treatments with 5 replications.

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**Place and Duration of Study:** The experiment was carried out from March to November 2016, in a greenhouse of Embrapa Eastern Amazon in the municipality of Belém, State of Pará, Brazil, located at the geographic coordinates 01° 27' of South Latitude and 48° 30' of West Longitude.

**Methodology:** The following parameters were evaluated: predawn water potential ( $\Psi_{pd}$ ), water potential of xylem ( $\Psi_x$ ), stomatal conductance (GS), transpiration (E), photosynthesis (PS), internal carbon concentration (Ci), internal and external carbon ratio (Ci/CA), chlorophyll a (Chl a), b (Chl b), total (Chl total), anthocyanin (ANT), carotenoids (CAR), instantaneous carboxylation efficiency (ICE), and water-use efficiency (WUE). The variables were submitted to multivariate analysis.

**Results:** Two distinct groups were resulted. Group 1 retained the treatments with water deficiency and group 2 retained the control treatments. This result occurred because the water conditions were different. The MC1 was influenced by PS because photosynthetic pigments are essential for photosynthesis. PS, GS, Chl total, Chl a, CAR, Ci, Ci/CA,  $\Psi_{pd}$ ,  $\Psi_x$ , E, and ICE resulted with greater intensity in the control treatments, and with less intensity in the genotypes submitted to water deficiency.

This behaviour is because of water deficiency that reduces photosynthesis. The MC2 was influenced by Chl b, ANT, and WUE. In water deficiency, the distinction in the physiological behaviour of cupuaçu tree progenies occurs in relation to the progenies that did not undergo water stress.

**Conclusion:** The cupuaçu tree progenies under water restriction condition have a positive effect on water-use efficiency.

*Keywords: Physiological behaviour; abiotic stress; genotypes; multivariate analysis.*

## 1. INTRODUCTION

Abiotic stresses in plants are one of the subjects that have aroused the interest of research due to the constant search for cultivars that are more productive when submitted to inadequate conditions for the cultivation [1]. According to the same authors, the stresses originating from water deficit and saline soil are the most studied due to the intense impact of these stresses on plant growth and productivity. Thus, the water limitation condition is responsible for several plant disorders due to the reduction in the cellular turgor, which is important for cell metabolism [1]. Furthermore, agricultural production is severely affected by water deficiency, as this is a limiting abiotic factor for agriculture [1,2], because this stress affects the absorption of water and essential chemical elements, which is absorbed by the plant [3]. Hence, water stress causes negative changes in plant metabolism [4].

With water stress, the plant may have the stomatal conductance impaired, causing losses in the photochemical stage of photosynthesis, resulting in the decrease of the plant biomass and imbalance in the antioxidant defence, which will lead to the oxidative stress of proteins, of membrane lipids and also of cell organelles [4]. According to Ashraf et al. [5] there are some plants that are able to develop morphological, anatomical, physiological, biochemical, and molecular mechanisms that help them to become

tolerant or adapted to thrive in water restriction. Thus, the intensity, duration, and progressive rate of water deficiency regulate the response of plants submitted to water stress, which are based on a set of physiological and cellular adaptations [6].

Water deficiency is undoubtedly one of the abiotic factors that limit agricultural cultivation on the planet [3] significantly. There are several studies on this subject [7-10]; however, research that addresses this issue is still necessary to obtain a better understanding of the mechanisms, which are used by plants for their adaptation to water-restricted sites [11,12]. Also, research on this subject has been receiving special attention by agronomists and physiologists, since it is important for the growth and agricultural production of plants [13], and also to the fact that food production in the world may be impaired by climate change. Therefore, studies aimed at understanding the physiological mechanisms of arboreal plants, as well as understanding the interactions of plant species with the environment, are essential [14]. To better understand the tolerance mechanisms to water restriction in plants, aspects related to the biochemistry of cells, as well as their morpho-anatomical characteristics, more research should be done in order to expose the associations that lead to a better tolerance of dry environments, favouring the agronomic improvement of plants [4,15]. It was also known that there could be

many genetic and environmental factors regarding the improvement [41].

The Cupuaçu tree [*Theobroma grandiflorum*] is a very important arboreal plant in the Amazonian region. Its pulp is used for the preparation of juices, candies, cakes, creams, bonbons, ice creams, liqueurs, and also dairy drinks [16]. According to these authors [16], the oil extracted from the seed is widely used in the cosmetics industry, and also to make the *cupulate*, which is a product similar to chocolate. Moreover, it is used in agroforestry systems in consortium with other economic expression cultivations of the Amazon.

Considering this context, this study tests the hypothesis that water deficiency affects the physiological behaviour of cupuaçu tree progenies. The objective of this study was to investigate the physiological behaviour of cupuaçu tree progenies submitted to water deficiency.

## 2. MATERIALS AND METHODS

### 2.1 Location and Experiment Conduction

The experiment was carried out from March to November 2016, in a greenhouse of Embrapa Eastern Amazon in the municipality of Belém, State of Pará, Brazil, located at the geographic coordinates 01° 27' of South Latitude and 48° 30' of West Longitude.

The climate of the region where the experimental area is located, according to the *Köppen* classification, is Afi, with an average annual temperature of 26°C and an average annual precipitation of 2,754.4 mm, occurring mainly during rainy season from December to May, and a less rainy season from June to November [17].

Seven Cupuaçu tree progenies were used, originating from seeds of parental clones of cultivar BRS Carimbó (Table 1).

The seedlings were prepared from seeds taken from ripe fruits, from open pollination, whose plantation is located in the experimental orchard of Embrapa Eastern Amazon, located in the municipality of Tomé Açu, State of Pará. Two fruits were harvested from each of the genotypes of cupuaçu tree, each fruit being characterised as to the weight of the fruit, of the peel, of the pulp, of the seeds and the fibre. The length and diameter of the fruit and the number of normal and withered seeds were measured. The seeds were manually pulped and later the sowing was carried out in a seedbed, containing tanned sawdust as substrate.

Twenty-two days after sowing, when the seedlings were in the stage before the opening of the first pair of leaves, the progenies were replanted into plastic bags, measuring 20 x 45 cm, and filled with 8 kg of substrate. Soil samples were collected from the plastic bags to perform a soil chemical analysis [18] from 0 - 20 cm depth (Table 2). The characterisation of the soil texture was performed using the granulometric analysis in 0 - 20 cm depth [18], thus, the soil presents 100 g kg<sup>-1</sup> of clay, 455 g kg<sup>-1</sup> of coarse sand, 327 g kg<sup>-1</sup> of fine sand and 118 g kg<sup>-1</sup> of silt.

Afterwards, the randomisation and distribution of the seedlings were carried out in the stand where they stayed to grow and to acclimatise in the greenhouse. The irrigation in the first two months of the experiment was carried out by a micro-sprinkler with a flow rate of 40 l/h, activated daily for 30 minutes. At the end of this period, irrigation was done manually, applying daily 300 ml of water per seedling to maintain the soil moisture near to the field capacity. From the eighth month,

**Table 1. Clones of origins of the evaluated progenies and their respective ancestry and provenances - Embrapa Eastern 2016**

Clone	Ancestry	Place of origin of the mother	Place of origin of the father
32	174x186	174: Coari – AM	186: Codajás – AM
42	186x434	186: Codajás – AM	434: Muaná – PA
46	186x215	186: Codajás – AM	215: Manacapuru – AM
47	186x1074	186: Codajás – AM	1074: Itacoatiara – AM
57	186x513	186: Codajás – AM	513: Oiapoque – AP
215	Primary	215: Manacapuru – AM	-
1074	Primary	1074: Parintins - AM	-

source: *embrapa eastern amazon.*

**Table 2. Chemical characterisation of the substrate**

Depth	MO	N	pH	P	K	Na	Ca	Ca+Mg	Al	H+Al
cm	g kg <sup>-1</sup>	%	H <sub>2</sub> O	..mg dm <sup>-3</sup> ..		.....cmol <sub>c</sub> dm <sup>-3</sup> .....				
0-20	25.81	0.88	6.2	374		504	2.6	4.5	0.1	1.49

source: *embrapa eastern amazon.*

the plants were submitted to two water regimes: one with water restriction and another without restriction (control). The irrigation was suspended for 16 days in the plants submitted to water deficiency, preserving the control plants under daily irrigation with 300 ml of water per plant.

The experimental design was completely randomised, in a 2x7 factorial scheme (2 water regimes: with and without water deficiency and 7 cupuaçu tree genotypes), totalling 14 treatments with 5 replications, making a total of 70 experimental units, each composed of one plant/bag. The two water regimes (with water and without water deficiency) were analysed as factor A and as factor B (Progenies).

## 2.2 Determination of the Water Status of the Plant of Cupuaçu tree Progenies

The leaf water potential was evaluated before dawn, from 4:00 a.m. - 5:30 a.m. and at noon, from 12:00 p.m. - 1:30 p.m. A Scholander type pressure pump (model PmsInstrumentCo., Corvallis, USA) was used as described by Damatta et al. [19]. The liquid photosynthetic rate, stomatal conductance, transpiration rate, internal carbon concentration and internal and external carbon ratio were evaluated using the LI-6200 portable infrared gas analyser (Li-Cor), measured under favourable environmental conditions, between 8:00 a.m. and 10:00 a.m. From these variables, the water-use efficiency (WUE) ( $A/E$ ) [ $(\mu\text{mol m}^{-2} \text{s}^{-1}) (\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1})^{-1}$ ] and the instantaneous carboxylation efficiency (ICE) ( $A/C_i$ ) [ $(\mu\text{mol m}^{-2} \text{s}^{-1}) (\mu\text{mol mol}^{-1})^{-1}$ ] were calculated [20]. The evaluations were made in all plants of all treatments, being one leaflet per plant placed inside the chamber, always in the medium region of the fully expanded leaf. Each leaflet remained in equilibrium within the chamber for 1 to 2 minutes, recording the values for each reading of the plants. The determination of the chlorophylls *a*, *b*, *total*, anthocyanin and carotenoids were performed according to the method described by Sims and Gamon [21].

## 2.3 Data Analysis

The variability of the studied physiological variables was first evaluated through the

descriptive statistics, in which the values of the mean and standard deviation were calculated. After this analysis, the variables were submitted to the multivariate grouping analysis by hierarchical methods and main components.

For the grouping analysis, Ward's method [22] was performed, in which a matrix of similarity with the Euclidean distance and with the groups' connection was constructed. In the Ward method, the distance between the two groups is defined as the sum of squares between the two groups using all variables [23]. The Euclidean distance between the accesses for the set of evaluated biochemical variables was calculated, distinguishing among the studied factors and thus exposing the structure of the groups expressed in a dendrogram graph.

For the analysis of main components (AMC), the following variables were used: Anthocyanin (ANT), carotenoids (CAR), internal carbon concentration (CI), *total* chlorophyll (Chl *total*), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), stomatal conductance (GS), photosynthesis (PS), predawn water potential ( $\Psi_{pd}$ ), water potential of xylem ( $\Psi_x$ ), internal x external carbon ratio (CI/CA), leaf transpiration (E), water-use efficiency (WUE) and instantaneous carboxylation efficiency (ICE). Subsequently, the set of variables was dimensioned according to their characteristics to better visualise the relationship between the variables in coordinate axes. These new axes, the eigenvectors, called main components (MC) were generated by linear combinations of the original variables constructed with the eigenvalues of the covariance matrix [23]. The aim was to obtain a simpler and more parsimonious model, using the Kaiser criterion (1958), with eigenvectors above the unity (1). The analyses were performed in the STATISTICA 7.0 software (StatSoft, Inc., Tulsa, OK, USA).

## 3. RESULTS AND DISCUSSION

Fig. 1 shows the formation of 2 distinct groups. In group 1, the treatments with deficiency were retained and in group 2 the control treatments were retained. Group 1 and group 2 do not exhibit similar physiological behaviours (Fig. 1).

This result was due to the fact that the water deficiency conditions and the water supply conditions to which the plants were submitted are different, for the plants submitted to water deficiency have a low photosynthetic rate, the regulation of the stomatal opening and closing, and the transpiration and gas exchanges processes are compromised, resulting in a greater efficiency in water use, which may be confirmed by Fig. 2. Thus, the water condition is fundamental for the plants to perform their physiological and biochemical processes. To carry out the photosynthesis, water is necessary for the release of protons and electrons of the photochemical stage. It is also required to regulate the opening of stomata, which favour the absorption of carbon dioxide and also the mobilisation of photoassimilates [24]. Therefore, plant breeding is important to obtain plants that are more tolerant to water deficiency [10], making the study involving water stress fundamental. Hence, plants that are submitted to water deficiency present an imbalance between energy capture and metabolism, with a reduction in the photochemical reaction and an increase in energy dissipation [25].

The MC1 was influenced mainly by photosynthesis (PS), in which this variable presents negative eigenvectors, confirming the highest amount of total chlorophyll (Chl *total*), chlorophyll a (Chl *a*), carotenoid (CAR), internal carbon concentration (CI), internal x external carbon ratio (CI/CA) and instantaneous carboxylation efficiency (ICE), i.e., the higher the quantity of these variables, the greater the occurrence of photosynthesis (Figure 2), because photosynthetic pigments are necessary for the photosynthetic process, in which it is necessary that the pigments absorb solar energy for the photosynthesis to begin. Moreover, Chl *a* is essential for the conversion of solar energy into chemical energy and then the production of organic compounds (carbohydrates and amino acids, for example) and carbon dioxide fixation.

It is also possible to observe in the MC1 that the PS directly influences stomatal conductance (GS), predawn water potential ( $\Psi_{pd}$ ), the water potential of xylem ( $\Psi_x$ ) and leaf transpiration (E) (Fig. 2) in the control treatments. This result was possibly because, in the control treatment the cupuaçu tree genotypes expressed their physiological potential since they have enough water to supply their physiological demands. Thus, the plant naturally regulates the opening and closing of stomata, favouring transpiration

and the water potential of the plant. Besides, the increase of photosynthesis in Cupuaçu tree progenies in the control treatments is related to stomatal opening, because under conditions of water stress the photosynthetic process is reduced. With water stress, the stomata closes as a plant defence mechanism to prevent the loss of water by perspiration, thus maintaining the turgor of the cells [7]. Furthermore, photosynthesis may be limited when stomatal conductance decreases because the reduction of the availability of carbon dioxide (CO<sub>2</sub>) occurs in the leaf mesophyll, this process being affected by the reduction of internal carbon [26]. Hence, it is evident that water stress affects stomatal conductance and this condition may be an indicator of such stress [27].

It is also possible to observe in Fig. 2 that the PS, GS, Chl *total*, Chl *a*, CAR, CI, CI/CA,  $\Psi_{pd}$ ,  $\Psi_x$ , E and ICE occur with greater intensity in the control treatments of all genotypes, and occur with less intensity in the genotypes submitted to water deficiency. This behaviour is possibly because of water deficiency, that favours the reduction of photosynthesis. Wu et al. [28] observed that the levels of photosynthetic pigments, such as chlorophyll a, reduced under water stress conditions, thus reducing the photosynthetic process. In other studies, it was also observed that the photosynthetic rate was reduced in water restriction conditions [29, 30, 31]. Moreover, conditions of water stress may compromise the closing of stomata and transpiration, which will cause a reduction of photosynthesis [8].

The reduction of stomatal conductance in the cupuaçu tree progenies is probably due to the reduction of stomata opening, favouring a reduction in transpiration in the treatment under water deficiency. Thus, the reduction of stomatal conductance found in this study is in agreement with other researchers [25,32,33]. According to Hu et al. [7], plants, in general, promote the closing of stomata when they are submitted to a water restriction condition to avoid water loss, consequently decreasing stomatal conductance and transpiration.

The reduction in the transpiration process may also be caused by the reduction of hydraulic conductivity of the roots, causing a decrease in the leaf water potential [34]. In other studies, transpiration reduction was also observed in plants under water stress conditions [35,36].

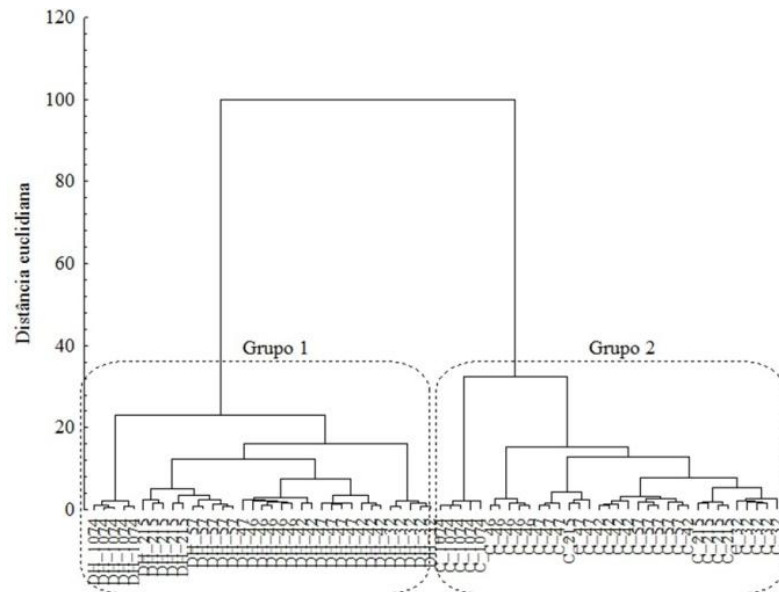
As shown in Fig. 2, CI and CI/CA ratio occurred with less intensity in cupuaçu tree genotypes under water stress conditions because under water deficiency conditions the rate of assimilation of carbon dioxide reduces and this may have occurred due to the decrease of availability of carbon dioxide from the internal part of the leaf, caused by the closing of stomata when the plant is in a limited water condition. Hence, the closing of stomata favours the increase of the diffusion resistance of the carbon dioxide to the catalytic site of the Rubisco enzyme, causing a negative effect on the intercellular carbon concentration [37]. The same behaviour was observed by other authors [38,26].

The MC2 was influenced by chlorophyll *b* (Chl *b*) and anthocyanin (ANT) (Fig. 2). These variables presented lower quantities in the treatments with water deficiency. The fact that Chl *b* has been influenced in the MC2 is because, Chl *b* is an accessory pigment, which does not perform the energy conversion function as Chl *a*.

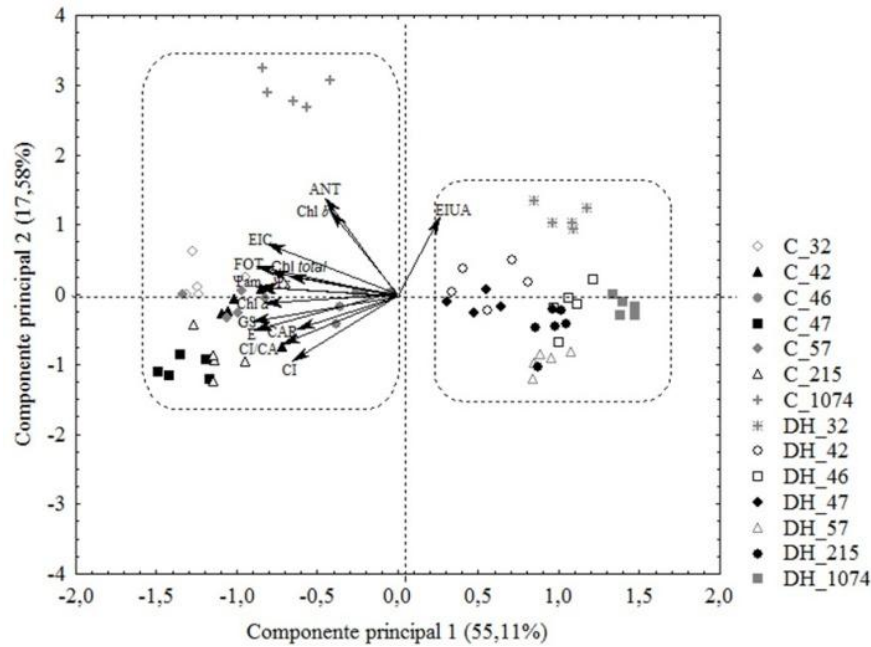
In the MC2, the water deficiency treatments in cupuaçu tree genotypes present a higher water-use efficiency (WUE) (Fig. 2). This behaviour probably occurred because the evaluated cupuaçu tree progenies may present

morphophysiological mechanisms that allow the development of these progenies under conditions of water stress and, consequently, may present mechanisms that elevate WUE. According to Sampaio et al. [39], due to water scarcity regarding global warming, it is necessary to use practices that allow the efficiency of water use or increase the resistance of crops to water stress. Therefore, the development of cupuaçu tree genotypes is very important for water stress conditions. Tosta [40], in his study on water stress and efficiency of water use by onion plants, observed that WUE or resistance to water deficiency was affected by the characteristics of the onion genotype when evaluated in various soil water conditions.

Regarding Table 2, it may be observed that the physiological variables CAR, CI, Chl *total*, Chl *a*, GS,  $\Psi_{pd}$ ,  $\Psi_x$ , CI/CA, E, and ICE are directly related to the PS, i.e., as these variables show higher expression in the cupuaçu tree genotypes, the increase of the photosynthetic process occurs. It is also observed that the variables ANT, Chl *b* and WUE are independent of MC1 in this study, i.e., they are not influenced by the variables retained in MC1 (Table 2). In addition, the variables ANT, Chl *b* and WUE are directly correlated, as shown in Table 2.



**Fig. 1. Dendrogram demonstrating the hierarchy of the groups of the aerial part (leaves) of the plants of *Theobroma grandiflorum* (Willd. ex. Spreng.) Schum submitted to water deficiency, resulting from the grouping analysis by the hierarchical method**



**Fig. 2.** Analysis of Main Components (AMC) of MC1 and MC2 with the following variables: Anthocyanin (ANT), Carotenoids (CAR), internal carbon concentration (CI), *total* chlorophyll (Chl *total*), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), stomatal conductance (GS), photosynthesis (PS), predawn water potential ( $\Psi_{pd}$ ), water potential at noon ( $\Psi_x$ ), internal x external carbon ratio (CI/CA), leaf transpiration (E), water-use efficiency (WUE) and instantaneous carboxylation efficiency (ICE) in the aerial part (leaves) of the plants of *Theobroma grandiflorum* (Willd. ex. Spreng.) *Schum* submitted to water deficiency.

**Table 2.** Correlation coefficient of the main components for the variables: Anthocyanin (ANT), Carotenoids (CAR), internal carbon concentration (CI), *total* chlorophyll (Chl *total*), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), stomatal conductance (GS), photosynthesis (PS), predawn water potential ( $\Psi_{pd}$ ), water potential of xylem ( $\Psi_x$ ), internal x external carbon ratio (CI/CA), leaf transpiration (E), water-use efficiency (WUE) and instantaneous carboxylation efficiency (ICE)

Biochemical variables	MC1 (55.11%)	MC2 (17.58%)
ANT	-0.46	<b>0.77</b>
CAR	<b>-0.63</b>	-0.28
CI	<b>-0.67</b>	-0.55
Chl <i>total</i>	<b>-0.82</b>	0.18
Chl <i>a</i>	<b>-0.84</b>	-0.07
Chl <i>b</i>	-0.42	<b>0.68</b>
GS	<b>-0.93</b>	-0.21
PS	<b>-0.90</b>	0.23
$\Psi_{pd}$	<b>-0.88</b>	0.05
$\Psi_x$	<b>-0.76</b>	0.17
CI/CA	<b>-0.73</b>	-0.41
E	<b>-0.90</b>	-0.29
WUE	0.26	<b>0.64</b>
ICE	<b>-0.82</b>	0.42

\*value relative to the percentage of variation of the original set of data retained by the respective main components. correlations in bold (>0.50 in absolute value) were considered in the interpretation of the main component.

The two-dimensional plane generated with the first two main components (MC) corresponds to 72.69% of the information contained in the original data: 55.11% in Main Component 1 (MC1) and 17.58% in Main Component 2 (MC2) when analysing the physiological parameters of cupuaçu tree progenies (Fig. 2, Table 2). These results are consistent with the criterion established by Sneath and Sokal [41], in which the number of MC used in the interpretation must be such that it explains at least 70% of the total variance of the data.

#### 4. CONCLUSION

In water deficiency, the distinction in the physiological behaviour of cupuaçu tree progenies occurs in relation to the progenies that did not undergo water stress, with the formation of two distinct groups in the multivariate grouping analysis.

The cupuaçu tree progenies under the condition of water supply have a positive effect on the physiological behavior, with an increase in the amount of anthocyanin, carotenoids, internal carbon concentration, total chlorophyll, chlorophyll *a*, chlorophyll *b*, stomatal conductance, photosynthesis, predawn water potential, water potential of xylem, internal x external carbon ratio, leaf transpiration and carboxylation efficiency; while, the cupuaçu tree progenies under the condition of water restriction have a positive effect on the water-use efficiency.

New studies are necessary for detail the enzymatic, molecular and genetic behaviour and of growth of the progenies in field conditions, with the aim to evaluate the development of these plants in non controlled conditions.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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