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Analysis of Leaf Pigments of SJ-02 Cocoa Seedlings Under Different Irrigation Depths and Cultivation Containers

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Abstract

The analysis of leaf pigments helps to understand the behavior of plant species under stress conditions. Thus, the objective of this study was to evaluate the amount of leaf pigments in SJ-02 cocoa seedlings under different irrigation depths and cultivation containers. The experiment took place at the Federal Institute of Espírito Santo, Campus Itapina, in Colatina, a city located in the northwest of the State of Espírito Santo, Brazil. A total of 144 seedlings were used, distributed in a completely randomized design, in a factorial scheme (6 × 3), where the first factor consisted of six different irrigation depths: 4; 6; 8; 10; 12 and 14 mm d^{-1} and the second one consisted of three different cultivation containers: polypropylene bags with dimensions of 10 × 20, 15 × 25 and 13 × 35, totalling 18 treatments, with eight repetitions. Seventy-four days after sowing, total chlorophyll content, flavonoid index and anthocyanin index were evaluated. There was no interaction between the factors studied. The 7 mm d^{-1} irrigation depth is the most suitable because it is practical in water management. Significance was not observed between the different cultivation containers.

Keywords: *Theobroma cacao* L., chlorophyll content, photosynthetic capacity

1. Introduction

Brazil stands out as the sixth largest world producer of cocoa (*Theobroma cacao* L.) with a total of 250,308 tons produced in 2018 (Agrianual, 2017; IBGE, 2019). Its almonds have high commercial value because they are used in the food industry in the production of chocolate, juices, jellies and creams, in addition to serving as raw material for the cosmetics industry (Almeida & Valle, 2007).

One of the main steps for the success of an orchard is the production of seedlings, as they are directly related to the productive performance of the adult plant, which can guarantee more excellent uniformity, rapid growth and early harvest (Franco & Prado, 2008; Costa et al., 2010)

About the cultivation containers, their use is closely related to the cost of seedling production, as it affects the amount of substrate and inputs to be used and requires physical space in the nursery and labor for handling (Queiroz & Melem Junior, 2001). Regarding the use of water in agriculture, it is known that under water stress, plants undergo morphophysiological changes, altering the opening and closing of the stoma, reducing the process of photosynthesis, cell turgor and limiting cell division and expansion, which interferes with the growth and in plant development (Taiz & Zeiger, 2013).

The determination of phenolic and secondary compounds such as the index of flavonoids and anthocyanins, in addition to the chlorophyll content present in plants can be a useful tool to understand the behavior of plant species under stress conditions (Berilli et al., 2016; Oliveira et al., 2020). However, few studies relate water management and the cultivation container with these compounds. Thus, facing the notorious importance of the cocoa crop, the objective of this study was to understand the behavior of the total chlorophyll content, flavonoid index and anthocyanin index in SJ-02 cocoa seedlings under different irrigation depths and sowing containers.

2. Method

The study took place at the Federal Institute of Espírito Santo, Campus Itapina, in Colatina, city situated in the northwest of the State of Espírito Santo, Brazil, located with geographical coordinates of 19°32′22″ south latitude and 40°37′50″ west longitude. In the period from October 1st to December 13th, 2018. The region presents, according to the Köppen classification, climate tropical Aw, with dry winter and predominance of summer rain (Alvares et al., 2014).

Six individual rooms with dimensions of 2.20 in length and 1.10 in width were set up where six GREEN MIST (NaanDanJain®) anti-drop nebulizers were installed in each room located 1 m above the seedlings and spaced 0.8 m apart. Irrigation was performed with centrifugal pumps of 0.5 hp, with service pressure of 2 kgf cm^{-2}, controlled individually and electronically, with irrigation distributed for 10 hours a day.

The design used was completely randomized in a factorial scheme (6 × 3), where the first factor was made up of six different irrigation depths: 4; 6; 8; 10; 12 and 14 mm d^{-1} and the second factor consists of three different cultivation containers, being polypropylene bags with dimensions of 10 × 20 (small), 15 × 25 (medium) and 13 × 35 (large). In total, 18 treatments were analyzed, with eight repetitions, totaling 144 plants in the whole experiment.

To prepare the seedlings, the different crop containers were filled with commercial substrate Tropstrato HT® Vegetables plus Osmocote Plus® 15-9-12 (3M) in the dosage of 12 kg/m^3 with a guarantee of 15% N (7% ammoniacal and 8% nitrate), 9% P2O5, 12% K2O, 1.3% Mg, 5.9% S, 0.05% Cu, 0.46% Fe, 0.06% Mn and 0.02% Mo. The seeds used (one seed per container with a depth of approximately 2 cm) were from SJ-02 cocoa obtained from fully ripe fruits, which had the mucilage removed by rubbing.

Seventy-four days after sowing, one leaf from each plant was evaluated, obtaining the total chlorophyll content (SFR-G and SFR_R), flavonoid index (FLAV) and anthocyanin index (ANT_RG and ANT_RB). All evaluations were carried out using a Multiplex® fluorometer (Force-A) model, in which the indices represented by the letter G indicate measurements obtained by the green light and the indices represented by the letter R by the red light.

All data were analyzed using the F-test at 5% probability. The effect of the influence of the irrigation depth on the evaluated characteristic was tested by regression analysis using the Student's t-test at 5% probability. When significant, the equation model that best represented the effect of the irrigation depth on the evaluated characteristic was adjusted. The maximum points were calculated using the primary derivative of the regression equation. The Tukey's average comparative test tested the effect of the different cultivation containers on the characteristics evaluated at 5% probability. Statistical analyzes were performed with the R software (R Core Team, 2020), using the data package ExpDes.pt version 1.2 (Ferreira et al., 2010).

3. Results and Discussion

Table 1 shows the summary in the experimental variance analysis obtained through the F-test at 5% probability. It is possible to observe that for all the studied characteristics, there was a significant effect related to the applied irrigation depth. There are no significant differences for the characteristics regarding the cultivation container used and no effect of the interaction between the irrigation depth factors and cultivation containers for all characteristics.

Table 1. Summary of analysis of variance with the source of variation (SV), degree of freedom (DF), average square (AS) and coefficient of variation (CV) for the characteristics of chlorophyll content (SFR_G and SFR_R), flavonoid index (FLAV) and anthocyanin index (ANTH_RG and ANTH_RB)

SV	DF	AS				
		SFR_G	SFR_R	FLAV	ANTH_RG	ANTH_RB
D	5	0.1611*	0.1373^{*}	0.0284^{*}	0.0047^{*}	0.0098^{*}
C	2	0.0654^{ns}	0.0377^{ns}	0.0068^{ns}	0.0007^{ns}	0.0034^{ns}
D×C	10	0.0592^{ns}	0.0316^{ns}	0.0031^{ns}	0.0011^{ns}	0.0033^{ns}
Res.	90	0.0664	0.0482	0.0024	0.0014	0.0019
Total	107					

Note. * Significant at 5% probability by the F test; ^{ns} not significant; D = irrigation depth; C = cultivation container; Res = residue.

The SFR_G and SFR_R indexes that represent the total chlorophyll content showed a quadratic behavior, with a maximum point of 2.16 and 1.86 in the irrigation depths of 7.28 and 7.01 mm d^{-1} and a determination coefficient (R^2) of 0.7013 and 0.6996, respectively. Chlorophylls are pigments directly responsible for the photosynthetic capacity of the plant, and the high efficiency of this process can reflect productive gains since there is a direct relationship between production and use of the radiation absorbed by them (Silva et al., 2014).

The analysis of the total chlorophyll content allows to infer about the photosynthetic apparatus of the plant, constituting an indispensable tool in the evaluation of the integrity of the cells, being a precise technique to identify plants that have tolerance to water stress conditions (Rong-Hua et al., 2006; Jabeen et al., 2008). It was possible to note that the smallest irrigation depths had a negative influence on the total chlorophyll content in the cocoa tree seedlings, with a gradual growth to the maximum point and from then on, the excessive amount of water available in the largest irrigation depths returned negative effect on seedlings.

Under water stress, a decrease in chlorophyll content is commonly observed, which consequently influences the photosynthetic system of the plant (Silva et al., 2014). In addition, the decrease in these photosynthetic pigments can lead plants to leaf senescence, reducing their life cycle and this reduction is directly linked to limited capacity and greater degradation of total chlorophyll contents, because under water stress conditions, plants alter their routes to avoid photoinhibition and photooxidation of the photosynthetic apparatus (Pincelli, 2010; Moro et al., 2015).

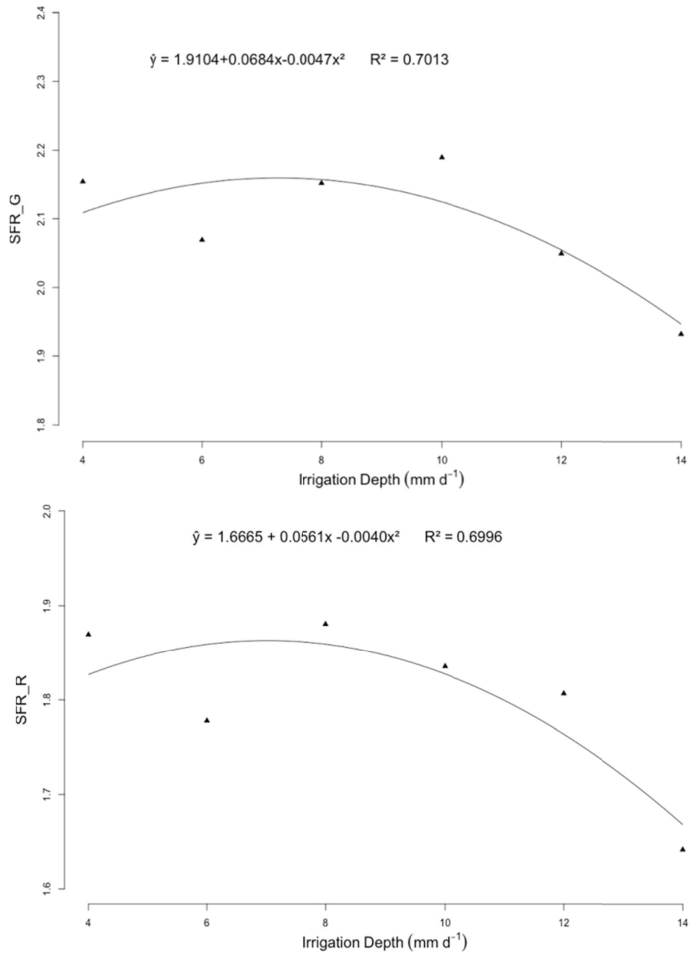

Figure 1. Effect of different irrigation depths on the total chlorophyll content (SFR_G and SFR_R) of SJ-02 cocoa seedlings

The flavonoid index (FLAV) had a quadratic adjustment, with the highest index of 0.017 being observed in the irrigation depth of 9.33 mm d^{-1}, with R^2 of 0.8742. Among the main functions associated with flavonoids, protection against environmental factors such as temperature changes, nutritional deficiency and solar radiation stands out (Castro et al., 2005). In physiological terms, under stress conditions, plants change their metabolism by allocating fixed carbon to secondary metabolites such as flavonoids at the expense of compounds such as cellulose, lipids and proteins (Abreu & Mazzafera, 2005; Kirakosyan et al., 2004).

In spite of little to be known about the influence of the water regime on the production of flavonoids in plants (Pacheco et al., 2011), under the conditions of the present study, it was observed that for the cocoa seedlings there was a gradual increase in the quality of flavonoids up to the 9.33 mm d^{-1} irrigation depth, from then on the quality of this metabolite decreased to the largest irrigation depths tested.

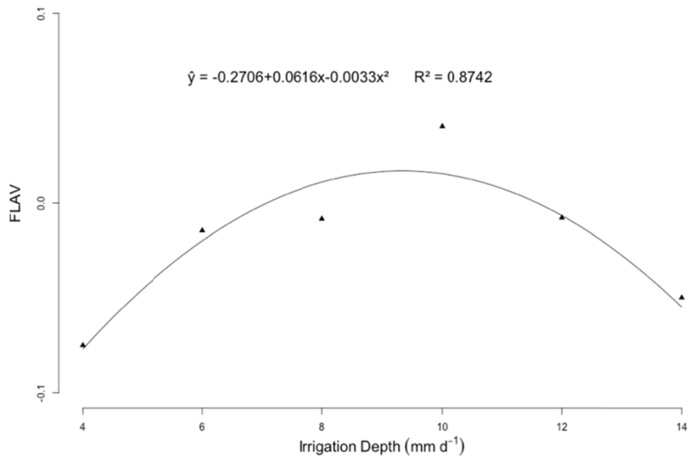

Figure 2. Effect of different irrigation depths on the flavonoid index (FLAV) of SJ-02 cocoa seedlings

Anthocyanins, represented by the ANTH_RG and ANTH_RB indexes, presented quadratic effects with a maximum point of 0.0092 and -0.513 in the irrigation depths of 10.05 and 9.3 mm d^{-1}, with R^2 of 0.8026 and 0.9166, respectively. These secondary compounds are the most relevant subcategory of flavonoids, is abundantly found in nature (Volp et al., 2008; Craft et al., 2012). Anthocyanins play several roles in plants such as protecting the action of ultraviolet rays, antioxidants, in addition to exercising defense against herbivores and assisting in the process of seed reproduction and dispersion (Lopes et al., 2007).

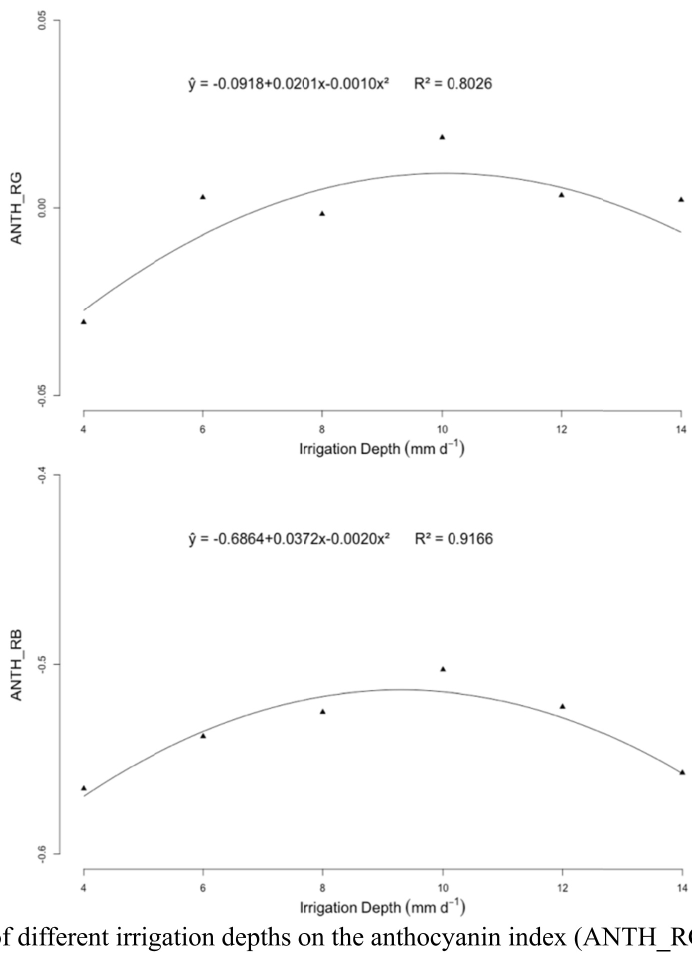

Figure 3. Effect of different irrigation depths on the anthocyanin index (ANTH_RG and ANTH_RB) of SJ-02 cocoa seedlings

Table 2 shows the averages of the values of the characteristics analyzed in relation to the different cultivation containers by the Tukey test at 5% probability. As already noted in the analysis of variance, there were no significant differences for any of the characteristics. Thus, the chlorophyll content, flavonoid and anthocyanin indexes were not influenced by the size of the cultivation container and, therefore, other characteristics of the containers must be taken into account since the size of the container interferes with the space to be used by the nursery, as well as the amount of substrate and labor may directly influence the production costs of the seedlings (Almeida, 2008).

Table 2. Average values of chlorophyll content (SFR_G and SFR_R), flavonoid index (FLAV) and anthocyanin index (ANTH_RG and ANTH_RB) of SJ-02 cocoa seedlings in three different types of cultivation containers

CONTAINER	SFR_G	SFR_R	FLAV	ANTH_RG	ANTH_RB
Small	2.0831a	1.7941a	-0.0347a	-0.0027a	-0.5463a
Medium	2.0527a	1.7741a	-0.0144a	0.0044a	-0.5305a
Large	2.1369a	1.8375a	-0.00833a	-0.0042a	-0.5283a

Note. Averages followed by the same letter between columns do not differ by Tukey's test at 5% probability.

Based on the results obtained in this study, it is possible to infer that both the excess and the lack of water negatively influence the amount of pigments present in the cocoa seedlings, with the irrigation depths between 7.01 and 10.05 mm d^{-1} providing higher values for all the analyzed characteristics. However, for the total chlorophyll content, a characteristic that reflects the photosynthetic capacity of the plant, the irrigation depths 7.28 and 7.01 mm d^{-1} were the most suitable. Thus, given the practicality of handling, it is recommended the 7.00 mm d^{-1}. Significance was not observed between the different cultivation containers attesting that the size of the container does not interfere with the pigment content present in the SJ-02 cocoa seedlings, thus, other characteristics such as production costs must be taken into consideration for the selection of the best cultivation container.

4. Conclusion

For the production of SJ-02 cocoa seedlings, there was no significant interaction between the factors of irrigation depth and cultivation container. The 7 mm d^{-1} irrigation depth, as it presents greater water management practicality and implies higher values of total chlorophyll content, is the most suitable. The size of the cultivation containers does not affect the pigment content present in the seedlings.

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