



INFLUENCE OF LIGHT ON SOME PHYSIOLOGICAL CHARACTERISTICS OF COFFEE (*Coffea arabica* L. cv. Caturra) IN NURSERY CONDITIONS

Influencia de la luz en algunas características fisiológicas del cafeto (*Coffea arabica* L. cv. Caturra) en condiciones de vivero

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ABSTRACT. The coffee tree is cultivated in the shade or in sunlight, hence the importance of studying its growth and physiological behavior in different light conditions. To contribute to that knowledge, in the National Institute of Agricultural Sciences (INCA) Cuba to 130 m s. n. m. has been made an experiment under nursery conditions with four light levels (100, 70, 50 and 20 %). The following indicators was evaluated: total dry mass and organs, leaf area, chlorophyll content, stomatal density and leaf anatomy. A sampling design was used for data processing and analysis of variance, confidence intervals and regressions were performed. The results showed that the positions grown with 20 % of light had higher values in terms of dry mass and leaf area. The largest contribution to the total dry mass was given by the leaves and roots; the specific leaf area was lower in the level of 100 % of light, followed by 50 % with significant differences. Stomatal density was higher in treating of 100 % of light, differing significantly from the others. Chlorophyll content was higher in the positions with less lighting, being the highest level of 50 % with a significant difference from the other treatments. These results indicate that morphological and physiological changes were produced in response to light restriction, which demonstrate the resilience of coffee to effectively utilize the available light.

Key words: coffee, chlorophyll, growth, lighting, shade

RESUMEN. El cafeto se cultiva, tanto a la sombra como a pleno sol; por ello, la importancia de estudiar su crecimiento y comportamiento fisiológico en diferentes condiciones de iluminación. Para contribuir a ese conocimiento se realizó un experimento en condiciones de vivero, con cuatro niveles de luz (100, 70, 50 y 20 %), en el Instituto Nacional de Ciencias Agrícolas (INCA), a 130 m s. n. m. Se evaluaron los siguientes indicadores: masa seca total y de los órganos, área foliar, contenido de clorofila, densidad estomática y anatomía de las hojas. Se utilizó un diseño de muestreo poblacional y para procesar los datos se realizaron análisis de varianza, intervalos de confianza y regresiones. Los resultados mostraron que las posturas expuestas a 20 % de luz, tuvieron valores mayores en cuanto a masa seca y área foliar. La mayor contribución a la masa seca total estuvo dada por las hojas y la raíz; el área foliar específica fue menor en el nivel de 100 % de luz, seguido del 50 % con diferencias significativas. La densidad estomática fue mayor en el tratamiento de 100 % de luz, diferenciándose significativamente de los demás. El contenido de clorofila fue mayor en las posturas con menor iluminación, siendo el más alto en el nivel de 50 % con diferencia significativa con respecto a los demás tratamientos. Estos resultados indican que se produjeron cambios morfológicos y fisiológicos como respuesta a la restricción de luz, que evidencian la capacidad de adaptación del cafeto para aprovechar eficientemente la luz disponible.

Palabras clave: café, clorofila, crecimiento, iluminación, sombra

INTRODUCTION

Environmental factors can cause different morphological, physiological and biochemical changes in crops, determining a variation in performance, because the interaction between these factors

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and physiological processes impact on improving production practices, to optimize photosynthesis and increase productivity of crops. Here, the shading is a useful strategy for growing shade tolerant species, in high irradiance areas (1). When the irradiance is reduced, evaporative losses of crop leaves and soil is also reduced, increasing the plant water and soil moisture^A.

In the genus *Coffea* greatly influences the intensity of light; the effect is manifested from changes in the vegetative growth to marked differences in yields, depending on the level of exposure to solar radiation (2). Although most coffee plantations are grown in full sun, studies suggest that the species must be cultivated in shade, mainly for the production of positions (3).

Despite accepting, generally, the importance of light and its management in coffee growing is still limited information concerning the behavior of coffee seedlings in different light environments, which limits the possibility of suggesting management. With this background, the embodiment of the present study was considered, with the objective of evaluating the influence of light on the physiological variables: dry mass, leaf area, chlorophyll content, stomatal density and anatomical structure of the leaves.

MATERIALS AND METHODS

An experiment was conducted at the premises of the National Institute of Agricultural Sciences (INCA), located in the municipality of San José de las Lajas, Mayabeque province, Cuba, at coordinates 23° 00' north latitude and 82° 12' in length west, at an altitude of 138 m and an average annual temperature of 23,6 °C.

The experiment was conducted under controlled conditions, 20 positions were placed with five pairs of leaves, under four light levels: 100 %, 70 %, 50 % and 20 %. The light intensity was reduced by placing nets of different density black polyethylene; these conditions were maintained until 105 days after starting treatment (DAT). Population sampling design was used, with a scheme field in strips, each strip representing a light level. To process the data analysis of variance were performed using Tukey's test at $p < 0,05$ for comparison of means (4); also for adjusting data regressions were performed using the Statgraphics Plus 5.0 software (5).

The confidence intervals analysis was performed using the Microsoft Excel program. Dry mass, leaf area, chlorophyll content, stomatal density and anatomical characteristics of leaves were evaluated.

The dry mass (g) was determined at 105 DAT, for which five plants were taken and each organ, which was dried in an oven of forced circulation at 80 °C to constant weight, it was then removed weighed in the balance SARTORIUS analytical, TE-214 model capacity 120 g, with an accuracy of 0,1 mg. With these data comparisons among treatments were performed with analysis of confidence intervals; and the dynamics of accumulation was studied, for which the data was fitted to a polynomial of the second degree exponential function. The following formula (6) was used to calculate the Absolute Growth Rate (AGR):

$$AGR = \frac{FDM - IDM}{t_2 - t_1}$$

Where:

MSF = final dry mass

MSI = initial dry mass

t = time

Leaf area (cm²) was measured at 105 DAT, using a portable leaf area meter ADC brand, model AM400. With these data the specific leaf area (AFE) was determined with the following formula (7):

$$AFE = \frac{AF}{FSAP} \text{ (cm}^2 \text{ G}^{-1}\text{)}$$

where:

LA = leaf area of the plant

DMLA = dry mass of leaf area

To determine chlorophyll content (g cm²) were performed every 15 days five measurements on leaves located in the central part of the position, for which a chlorophyll meter SPAD-502 Plus, Minolta brand, model was used standard; it calculates a numerical value which is proportional to the amount of chlorophyll in the leaf^B. The information was processed with analysis of confidence intervals in two stages: 60 and 105 DAT. To study the dynamics of growth, the data were fitted to a polynomial of the second degree exponential function.

Stomatal density (number per mm²) was determined by sampling the 105 DAT, which took the third pair of leaves of two positions for each treatment, forever sampling in the central area of the

^AGagliardi, S. *Intraspecific trait plasticity in coffee agroforestry systems of Costa Rica*. Tesis de Maestría, Universidad de Toronto, 2014, Canadá, 83 p.

^BSorbet, P.S. *Utilidad del medidor de clorofilas (SPAD-502®) en los programas de fertilización nitrogenada de trigo (Triticum aestivum L.) en regadío* [en línea] [Tesis de Grado], Universidad Pública de Navarra, España, 2013, p. 86, [Consultado: 1 de agosto de 2016], Disponible en: <<http://academica-e.unavarra.es/handle/2454/8785>>.

leaflet and on both sides of the midrib. Scraping the surface opposite to observe (8) was performed, once obtained the epidermal sheet was placed on a slide and stained with toluidine blue for five minutes. They were later made two washes of five minutes each, was placed again in slide with a drop of glycerin and covered with a cover slip for observation, held in an optical light microscope MOTIC and photographed with a camera the same brand, coupled thereto microscope.

For stomata counting, readings were performed on images taken microscope with 100x magnification. A total of 20 fields were taken for treatment. A stoma was considered when the two guard cells were present in the image. The data processing was done using a simple analysis of variance.

The anatomical characteristics of leaves were determined at 105 DAT, for which the sample of the third pair of leaves taken, counted from the top of 20 plants per treatment. Leaf samples of 0,5 cm², always from the central area of the leaflet and both sides of the midrib, which were placed in Eppendorf tubes for attachment to a solution of acetic 4 % formaldehyde (FAA) were taken at least 24 hours. A dehydration process of the samples in increasing concentrations of ethanol for 10 minutes, washing at each change was then performed. Once this step the samples were placed in a mixture of xylene/paraffin (v/v) and finally 100 % of paraffin for three hours; both steps in oven at 60 °C to maintain liquid paraffin. The samples were placed in paraffin in aluminum molds, for solidification at room temperature; cross sections of 10 µm with rotary microtome Minot were performed. Then it proceeded to dewax the cuts and they were stained with toluidine blue to 0,075 % (9).

The observations were made with an optical microscope MOTIC light and photographed with a camera of the same brand microscope coupled thereto. The images (100x and 400x) of the cross section of the leaf were exported to morphometric ImageJ program, where the linear measurement instrument was used. The thickness of the leaf blade, epidermis and parenchyma palisade and lagoon were determined perpendicular to the sheet and three points in the same. In the case of the skin provided by the center of epidermal cells, allowing measurements homogenize.

RESULTS AND DISCUSSION

total dry mass to 105 DAT showed the highest values in the treatments of 20 and 50 % light albeit with significant differences among them (Figure 1A); similar behavior was found in tobacco plants, where correspondence was shown this indicator growth with the intensity of illumination (2).

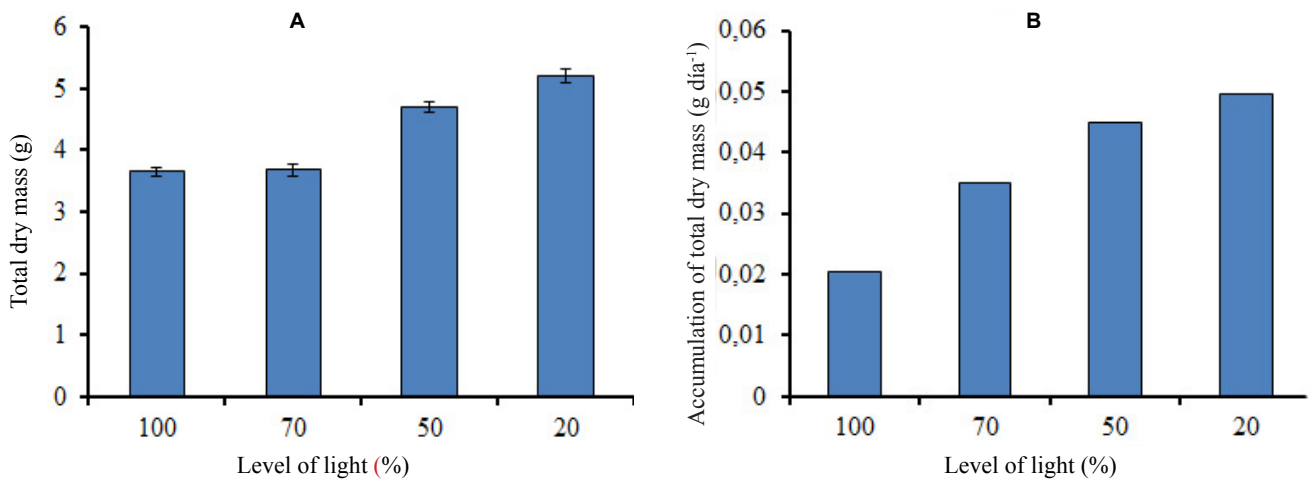
Also, the rate of accumulation of dry mass was increased as the light intensity of 0.021 g d⁻¹ in the sun to 0.050 g d⁻¹ under 20% light (Figure 1B) decreased. Values greater total dry mass in lower lighting levels showed increased efficiency foliage interception and utilization of available solar radiation during the growth cycle (10, 11).

By analyzing the dried dough bodies, it was observed that the highest values in sheets, was obtained in 20% treatment light; followed by treatment in full sun, with significant difference between them. The lowest values (1.82 and 1.92 g) occurred in 70 treatments and 50 % light, with no difference between them (Figure 2A). In other studies on coffee, it was found that higher values occurred in plants subjected to 50% light (12). Full sun treatment produced the highest values of dry mass stem, followed by 20 %, but differing from all other statistically (Figure 2B). As for the root, the highest values were presented for 20 %, followed by 100 % with differences among themselves and with other treatments (Figure 2C). This indicates that the major contribution to the dry mass, in all cases, was given by the leaves and root respectively (Figure 2D).

The response to treatments positions indicates that the greater the shade, higher dry mass production (12), which results in a tendency to achieve better performance as the light intensity decreases.

At 105 DAT, leaf area, reached the highest values in the light level of 20 %, with significant differences with other treatments; postures grown in full sun showed the lowest values, with differences versus restricted lighting (Figure 3A), producing a growth response due to the availability of radiation (13).

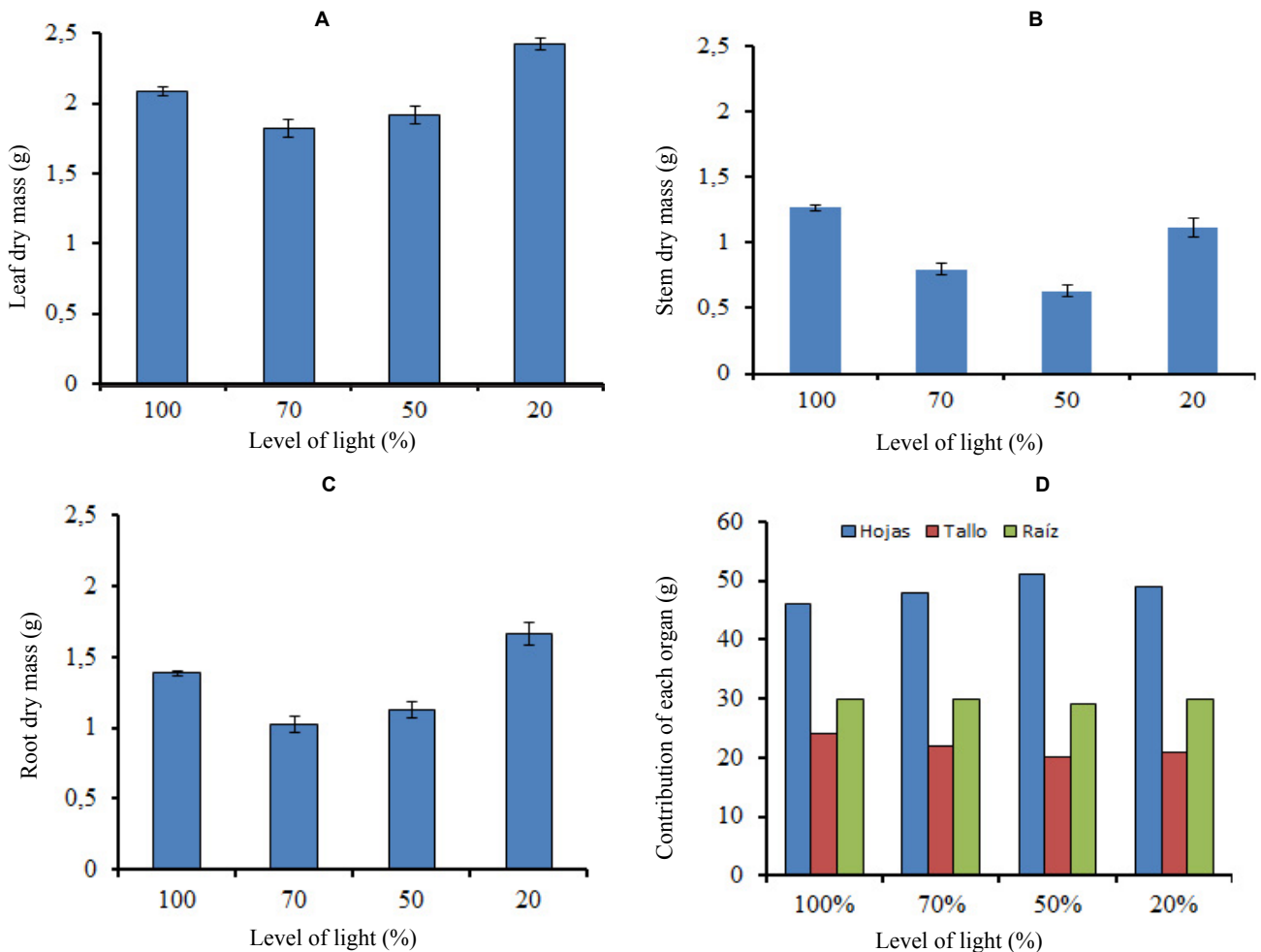
In a study in positions of coffee tree in Brazil, it was also found that positions grown in the shade, had greater leaf area growth linearly (10), indicating phenotypic plasticity of the positions to adapt to these ecophysiological conditions (14).



The vertical lines on the bars indicate the confidence interval (1- α = 0,05)

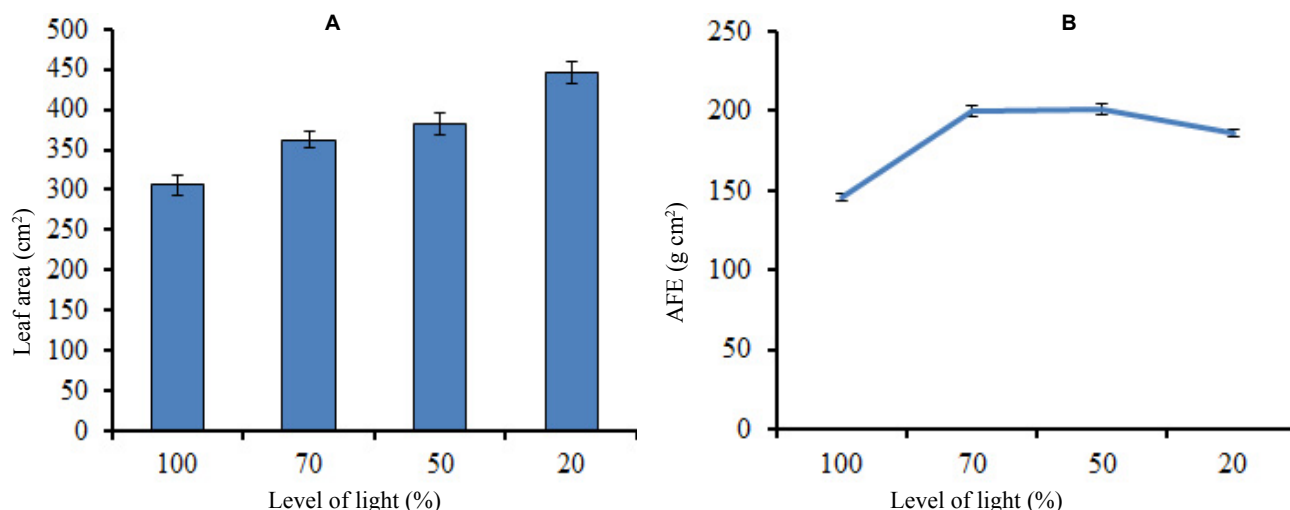
A. Total dry mass at 105 DAT B. TAC of total dry mass. INCA-Cuba

Figure 1. Dry mass in seedling coffee, grown in four levels of light



A. leaf dry mass B. dry mass stem C. dry mass root
 D. percentage contribution of each organ to the total dry mass. INCA-Cuba
 The vertical lines on the bars indicate the confidence interval (1- α = 0,05)

Figure 2. Dry mass of organs in coffee seedlings, grown with four levels of light at 105 DAT



A. Leaf area B. Specific leaf area, INCA- Cuba
The vertical lines on the bars indicate the confidence interval ($1-\alpha=0,05$)

Figure 3. Leaf area of the positions of the coffee tree, four light levels to 105 DAT

Specific leaf area (AFE) showed the lowest values in the treatment of full sun ($146.14 \text{ cm}^2 \text{ g}^{-1}$), with difference from others. In light restriction treatments, 20% had the lowest value ($186 \text{ cm}^2 \text{ g}^{-1}$), with differences compared to other treatments (Figure 3B). This implies that the positions of full sun treatment, but had lower leaf area and lower total dry weight, given the greater thickness of their leaves, lower surface invested by dry mass. In this regard, grown coffee plantations with low levels or relatively low light has been shown increased specific leaf area, and plant response to these conditions (15).

The behavior of the positions in the production of biomass and leaf area growth, was also expressed in the physiological response of the leaves, as the chlorophyll content was presented with higher values in the treatment of lower lighting, with significant difference full sun, increasing as the light linearly decreased to 60 DAT (Figure 4A).

A DAT 105 20% values were lower than light treatment of 50%, while the highest was chlorophyll content with significant difference, compared to the other (Figure 4B); this showed that chlorophyll increased as decreased light intensity to a certain limit, which has already begun to affect the selfshading, given the increase in leaf area, which, according to some authors (16, 17), generates some stress determining the drop in production of chlorophyll.

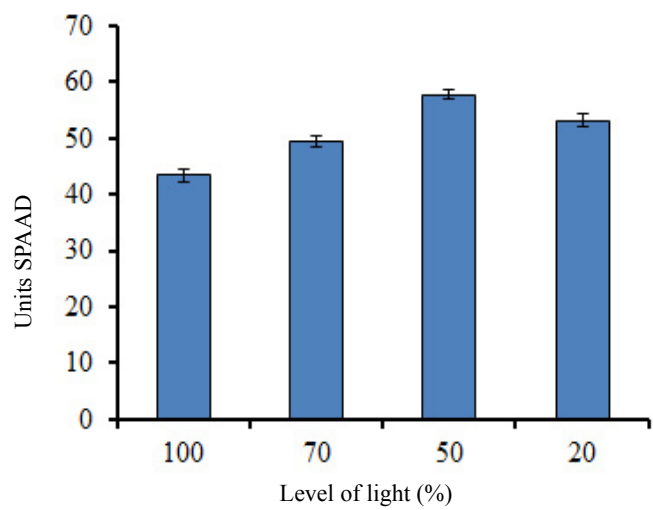
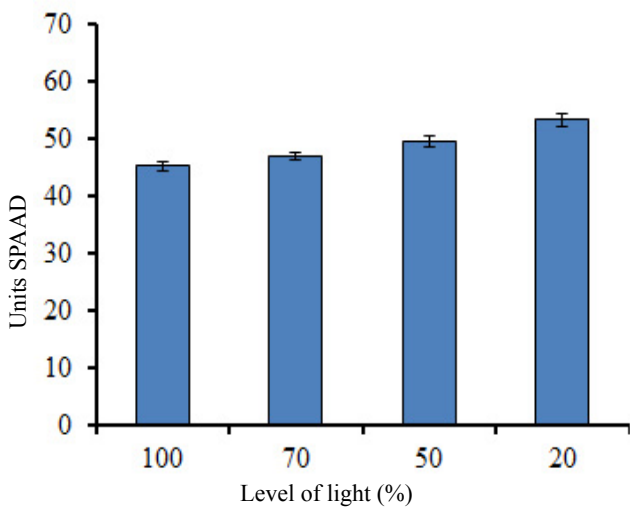
However, decreased chlorophyll content in the 20 % level of light, it was not enough to influence the decrease in dry mass.

Significantly response postures level 100%, which were relatively decreasing its chlorophyll content, with respect to the shaded; thus, 60 DAT this ratio was 85% compared to the highest value and the DAT 105 this relationship dropped to 75% relative to the highest value. In all cases there was a significant difference between the ends of shade.

Figure 4C shows the dynamics of chlorophyll content, which shows that treatments of 100 and 20% illumination, started to decrease its content to 75 DAT, while treatments of 50 and 70% began to decrease the 90 DAT. A DAT 105, lines 70 and 20%, joined, like chlorophyll content representing the end of the experiment.

In general, correspondence between the level of light and total chlorophyll content, which was also found in a study on coffee tree, where the leaves under irradiance of 15%, produced higher chlorophyll content expressed that they grew to full sun (19). In oil palm and *Apuntia cordifolia* similar behavior (20, 21) was also observed. These results may be related to the increase in the number of grana and thylakoids in chloroplasts, which makes more efficient uptake and transformation of light energy.

The low availability of light, induces leaf cells to increased photosynthetic pigments in order to increase the capacity of use of light and optimize photosynthetic efficiency (22), which results in some skill of physiological adaptation postures to capture and effectively utilize the limited light available (23).



A. to 60 DDT B. to 105 DDT C. dynamic
The vertical lines on the bars indicate the confidence interval (1- α = 0,05)

Figure 4. Chlorophyll content in SPAD units in coffee tree stands, in nursery with four levels of light

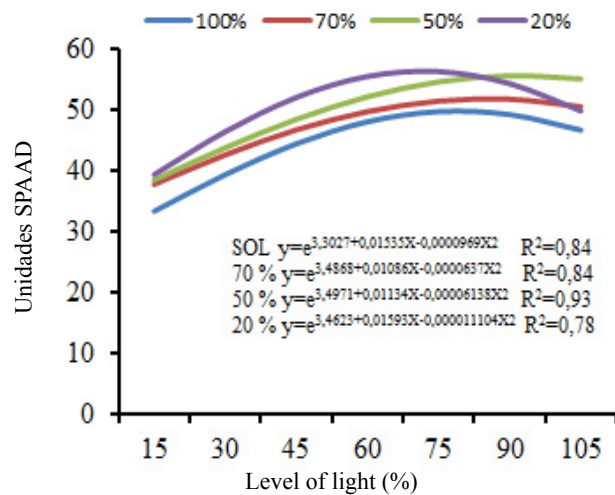


Table. Stomatal density of coffee seedlings to 105 DAT, cultivated with four light levels, INCA-Cuba

Level of light	Stomatic density (stomata mm ²)
Full sun	207,25 a
70 %	144,9 b
50 %	136,2 b
20 %	100,0 c
ES±	8,4*

Means with no common letter in the same column are significantly different at P ≤ 0.05, according to Tukey test (4)

It has also been studied that diffuse light, which could well be the effect of the mesh placed as a shadow, is distributed more evenly and increases processing capacity of energy for the most efficient use of this type of light, make leaves through their pigments (24).

The results showed that the leaves exposed to the sun, had a higher average number of stomata per leaf area, relative to the lower light levels, it has also been found in other studies on coffee (2); likewise, between plants grown with limited light, 70% and 50 % did not differ among themselves, but 50 % showed differences with 20%, which showed lower values (Table). In studies of tree and shrub species in the sun and in the shade, results increased frequency of stomata stomatal index point and by area of leaves exposed to the sun (25); on the other hand, in some species of tropical trees and Amazon, considering the sampled strata, it was determined that in the upper strata, where there was more light, stomatal density was higher (26, 27).

The values of stomatal density affected the stomatal index as they kept correspondence in behavior, being the highest value of the leaves exposed to sun and 70 % light and level 20 % lower values, with significant difference with full sun and 70 % (Table).

The leaves were presented thinner in plants grown at lower light intensity, with significant difference from floods to 100% light, treatments of 50 and 20% light, had the lowest values, without significant differences between them (Figure 5A). This behavior occurs because the lower the light intensity, the sheets expand its surface, to capture more light and thus are thinner.

The difference in thickness was related to the thickness of the cloud, as both the palisade parenchyma, as the lagoon, were thicker on leaves were at higher light intensity (Figure 5B and C). This became more visible in the cross section of the blade (Figure 6) showed differences in the thickness of the epidermis of the leaf blade, parenchyma palisade and spongy parenchyma, for each treatment.

In this regard, a sheet celtidácea found that the shade were thinner than those exposed to the sun (25); Likewise, in coffee plantations, it was observed that the more shade the leaves were thinner (15).

Generally, it was observed that the light directly influenced the growth of the positions and their physiological behavior. Lower lighting treatments obtained the highest values of dry mass, leaf area and total chlorophyll content, showing a close correspondence between these indicators; also the highest content of dry mass was given by the foliar part which presented a range of 46 to 51 % in treatment.

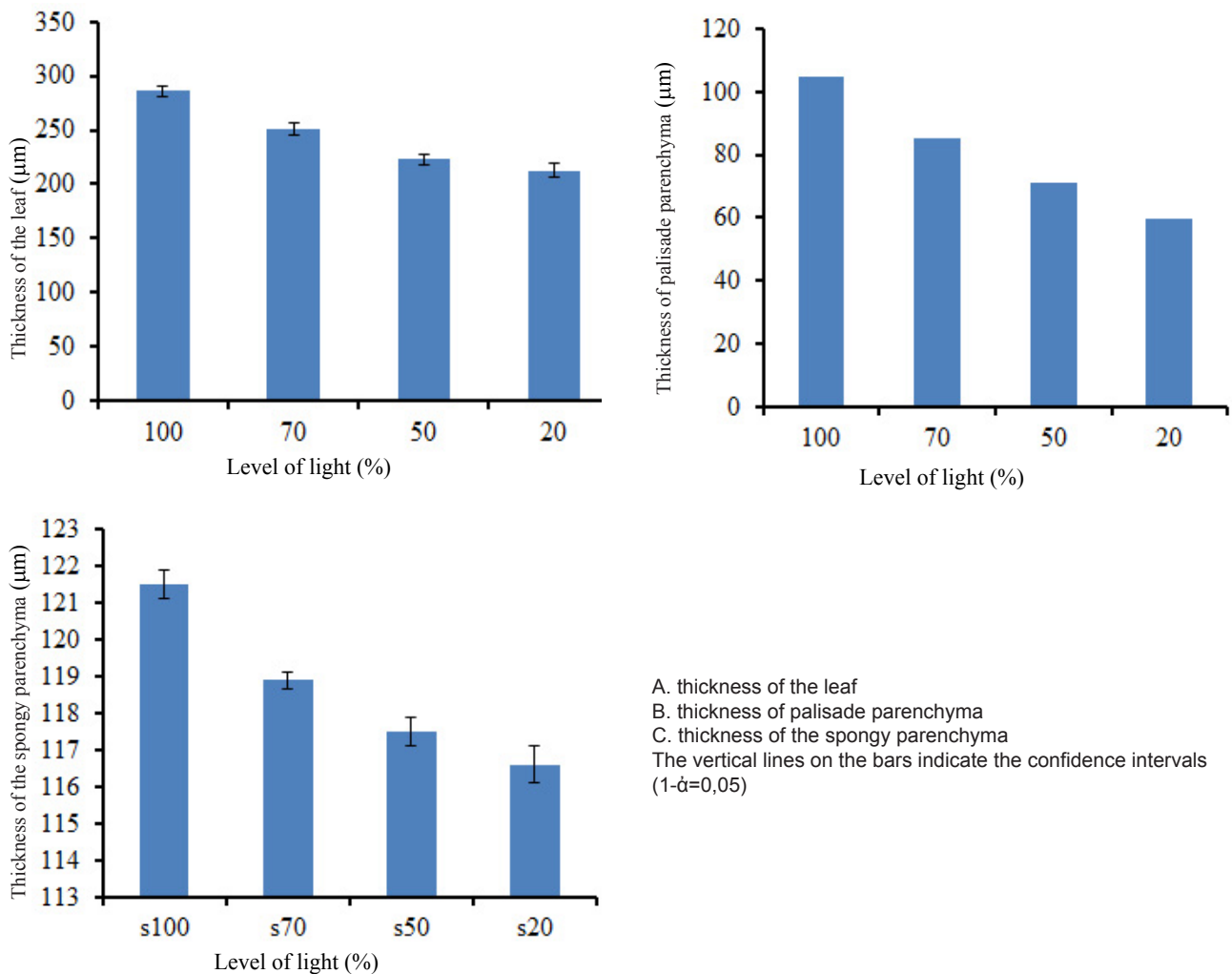
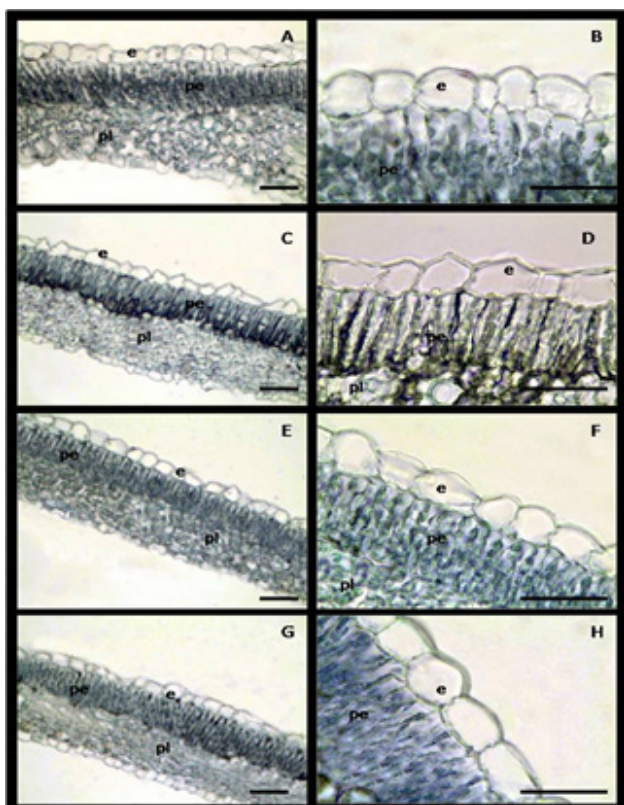


Figure 5. Anatomical structure of leaf coffee seedlings, grown with four light levels



e: epidermis pe: palisade parenchyma
 pl: parenchyma lagunar
 The horizontal bar represents 80 microns

Figure 6. Cross section of coffee leaves (*Coffea arabica* L.) plants under the sun (A, B); 70 % (C, D); 50% (E, F) and 20 % (G, H) of light observed microscopically at 100x and 400x

CONCLUSIONS

- ◆ The application of treatments found that stomatal density was higher, as was increasing the illumination level, leading to the conclusion that this behavior was given by the influence of light on the size of the leaves.
- ◆ It was evident that the greater the light intensity, the thickness of the leaf was higher, determined by the fastest growing palisade parenchyma and spongy parenchyma.

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