https://doi.org/10.1590/0034-737X2024710051
ISSN: 2177-3491

# Resilience to water deficit of coffee seedlings produced through cuttings and somatic embryogenesis

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**Editors:**
Ricardo Antonio Ayub
Marihus Baldotto

**Submitted:** March 16th, 2023.
**Accepted:** October 7th, 2024.

## ABSTRACT

Information on *Coffea arabica* L. vegetatively propagated during crop establishment is still scarce. Knowledge on the anatomical and physiological adaptation of these types of plants in the crop formation phase, in conditions of water deficit is important. The objective of this work was to understand the anatomical and physiological adaptations of plants derived from cuttings and somatic embryogenesis as resilience to water deficit in the implantation phase of the crop. Both types of plants were submitted to 20%, 40%, 60%, 80% and 100% of water available in the soil, in a controlled environment, for 153 days. The design used in this experiment was the randomized blocks with five replications. Physiological characteristics (photosynthetic activity, stomatal conductance, transpiration and instantaneous carboxylation efficiency) and anatomical characteristics (palisade parenchyma thickness and stomatal density) were evaluated. Both types of plants are resilient to water deficit in the planting phase of the crop. Cutting plants have greater photosynthetic activity and palisade parenchyma thickness with greater growth potential. In general, somatic embryogenesis plants have higher stomatal density. Plants of both types of seedlings have lower values of transpiration, stomatal conductance and $CO_2$ assimilation under water deficit conditions, but with higher stomatal densities, as an adaptation response.

**Keywords:** anatomy; cloning; early development of coffee; physiology.

## INTRODUCTION

New techniques for vegetative production of *Coffea arabica* L., such as somatic embryogenesis, have been made available to society, but there are still few works that provide anatomical and physiological results for greater knowledge of the adaptation of these types of seedlings in field conditions. Cloning in *Coffea arabica* L. is still little used because it is an autogamous species that propagates through seeds with the traditional option and cuttings as the most used option in *Coffea canephora* Pierre.

Nevertheless, the asexual production of seedlings can be interesting for the multiplication of high productivity materials, resistant to pests and diseases and with good drinking quality, in less time when compared to conventional breeding.

There are many doubts about the use in large-scale plantings of seedlings by cuttings and, mainly by somatic embryogenesis, and there is a need to better understand the anatomy and physiology of these plants, especially in conditions of water deficit.(1)

Knowledge of the anatomical and consequently physiological differences of the seedlings produced through cuttings and somatic embryogenesis is important as the Leading coffee producing regions in Brazil have suffered long periods of drought, as a result of global warming, a fact that tends to intensify over time, causing an imbalance between periods of excessive rainfall or intense droughts.(2)

The environmental variations to which plants are subjected have a direct influence on their leaf anatomy, and consequently their physiological activities, impairing-productivity particularly in the planting phase of the crop, when water deficits are frequent.(3)

In conditions of water deficit, losses in productivity and fruit quality may occur(4), changing the metabolism, modification the morphological, physiological and biochemical processes.(5,6) Thus, plants undergo physiological, morphological and biochemical modifications/adaptations as a response mechanism, to improve the efficiency of soil water extraction and improve water use efficiency.(7)

Among the modifications/adaptations of plants under conditions of water deficit, leaf anatomy plays an important role with variations such as: greater thickness of the leaf blade, greater number of conducting vessels and changes in stomatal dimensions.(8)

Thus, the anatomical and physiological characteristics of coffee plants have been used to characterize coffee plants under different growing conditions(9-14) with consistent and significant results.

Under conditions of water deficit, coffee plants initially close their stomata, thereby causing a reduction in transpiration, with a consequent reduction in photosynthesis and production of photoassimilates.(15) However, works that associate anatomical and physiological changes to water deficit in plants derived from cuttings and somatic embryogenesis are still scarce in the literature, making it difficult to develop more efficient management techniques for these types of seedlings and their resilience.

As a result, the objective of this work was to compare the anatomical and physiological adaptations of plants derived from the process of cuttings and somatic embryogenesis as resilience to water deficit in the crop establishment period.

## MATERIAL AND METHODS

### *Experiment location and description of plant material*

The experiment was carried out in a greenhouse at the Federal University of Lavras, state of Minas Gerais, from May 01 to September 30 and the minimum, average, maximum temperatures and relative humidity inside the greenhouse were monitored throughout the period of the experiment (Table 1).

Two types of coffee seedlings were used in the experiment. The first type of seedling was seedlings produced by somatic embryogenesis, with the genetic material being an F1 hybrid obtained by crossing the cultivars Acauã and Catucaí 785-15. To produce these seedlings, completely

**Table 1:** Temperature (T) and relative humidity (RH) in the greenhouse over the experiment

| Month | Minimum T (°C) | Mean T (°C) | Maximum T (°C) | Mean RH (%) |
|---|---|---|---|---|
| May | 9.8 | 20.4 | 37.5 | 68.8 |
| June | 10.5 | 20.2 | 39.0 | 69.7 |
| July | 9.2 | 19.0 | 38.3 | 69.2 |
| August | 7.5 | 21.2 | 40.7 | 57.7 |
| September | 8.9 | 23.5 | 45.1 | 56.5 |

expanded and healthy leaves were collected from the mother plant. Subsequently, the leaves were plucked and the leaf explants transferred to culture medium to obtain embryogenic callus and embryos. After obtaining the embryos, they were transferred to bioreactors for development. The pre-germinated embryos were transferred to trays with substrate, and later to tubes. In the tubes, the plants remained until the development of three pairs of fully expanded leaves, under 50% shade. After the development of three pairs of leaves per seedling, they were acclimatized and transferred to the experiment.

The second type of seedling was seedlings produced by cuttings, being used as genetic material to cultivate Siriema. To produce the seedlings, semi-hardwood cuttings were extracted from plagiotropic branches of the mother plants, measuring around five centimeters. Then, after hormonal treatment, the cuttings were transferred to tubes with substrate and kept in a climate-controlled greenhouse for rooting and growth of branches and leaves. After the development of three pairs of leaves per seedling, they were acclimatized and transferred to the experiment.

### *Soil used in the experiment and fertilization*

Vases a capacity of 20 liters were used, placed on 0.8-m high benches, filled with soil that had the following characteristics: pH (water) = 5.2; P (Mehlich-1) = 1.42 mg $dm^{-3}$; K = 14 mg $dm^{-3}$; Ca = 0.2 cmolc $dm^{-3}$; Mg = 0.1 cmolc $dm^{-3}$; Al = 1 cmolc $dm^{-3}$; H+Al = 12.28 cmolc $dm^{-3}$; Sum of Bases = 0.34 cmolc $dm^{-3}$; t = 1.34 cmolc $dm^{-3}$; V = 2.66%; Aluminum Saturation = 74.63%; Organic Matter = 4.29 dag $kg^{-1}$; Prem = 3.07 mg $L^{-1}$ and sand, silt and clay contents of 270, 30 and 700 g $kg^{-1}$, respectively.

The soil was corrected to 60% base saturation using 80% PRNT limestone containing 40% calcium oxide and 12% magnesium oxide. Healthy and acclimatized seedlings were used (cuttings and somatic embryogenesis), with six pairs of leaves, produced in 300-ml tubes. The application of fertilizers in the transplanting to the pots and for the topdressing fertilization, were carried out according to the recommendations of fertilization for pots of Malavolta.[16] After transplanting, all pots were maintained with 100% of water available in the soil, for thirty days, until the full establishment and uniformity of the plants, so that the application of treatments could then begin.

### *Experimental design*

The experimental design used was randomized blocks with five replications, in a 2 x 5 factorial scheme (2 types of seedlings and 5 irrigation levels). Each repetition was represented by a vase with one plant each. The types of seedlings used were: somatic embryogenesis, and cuttings. The second factor was represented by five levels of irrigation, maintaining the soil at 20%, 40%, 60%, 80% and 100% of available soil water.

### *Irrigation management methodology*

To maintain soil moisture, the soil water retention curve was characterized in the laboratory, using a Richards pressure chamber. The retention data obtained were adjusted according to the methodology proposed by Van Genuchten.[17]

For soil moisture control, tensiometers installed in the plots (pots) corresponding to 100% water available in the soil were used, which served as a parameter for the other irrigation levels. Four batteries of tensiometers were installed in four vessels, each battery containing two tensiometers: one installed in the upper layer (upper 50%) and the other in the lower layer (bottom 50%).

Through the retention curves, the depth to be applied in each plot was determined, using a specific spreadsheet for the calculations. The irrigations were carried out on Mondays, Wednesdays and Fridays, done manually with the aid of a graduated cylinder, as shown in Table 2.

### *Assessments carried out in the experiment*

At the end of the experiment, photosynthetic activity, stomatal conductance, transpiration and instantaneous carboxylation efficiency were evaluated, the latter given by the photosynthetic activity/intercellular carbon concentration

**Table 2:** Total amount of water applied in the treatments

| | Percentage of Available water in the soil | | | | |
|---|---|---|---|---|---|
| | **100%** | **80%** | **60%** | **40%** | **20%** |
| Total applied volume (L $vaso^{-1}$) | 14.5 | 11.6 | 8.7 | 5.8 | 2.9 |
| Equivalent depth (mm)[(a)] | 253.8 | 203.0 | 152.3 | 101.5 | 50.8 |
| Monthly Mean (mm)[(b)] | 50.8 | 40.6 | 30.5 | 20.3 | 10.2 |

[(a)]Accumulated volume of a vase extrapolated to equivalent soil surface. [(b)]Average applied in each month considering five months of evaluations.

ratio. These evaluations were performed using a portable infrared gas exchange analyzer (IRGA), with an artificial source of photosynthetically active radiation, in a closed chamber, fixed at 600 µmol of photons $m^{-2}$ $s^{-1}$ (Blue + Red LED LI-6400 -02B, LI-COR, Lincoln, NE, USA). The evaluations were performed between 9:00 a.m. and 11:00 am (solar time) on a typical sunny day, on a fully expanded leaf with no symptoms of damage (pests or diseases) per plant.

In addition to the physiological characteristics, the stomatal density and the thickness of the palisade parenchyma were Evaluated by collecting fully expanded leaves from the third pair of plagiotropic branches in the middle region of the plants. Immediately after collection, the leaves were fixed in F.A.A. 70[18] and after 72 hours they were placed in 70% ethanol ($vv^{-1}$) in order to preserve the material. Cross-sectional cuts were made using an LPC tabletop microtome, to assess the thickness of the palisade parenchyma, and paradermal cuts (freehand) to quantify the number of stomata. The sections were submitted to clarification with laboratory sodium hypochlorite (1.25% active chlorine), triple washing in distilled water, staining with safrablau solution (0.1% astra blue and 1% safranin in a 7:3 ratio) for sections sections and 1% safranin for paradermal sections. Subsequently, semi-permanent slides were prepared with 50% glycerol, observed under an optical microscope and photographed with a digital camera. The generated images were analyzed using the UTHSCSA-Imagetool software.

### *Statistical analyzes*

Data were submitted to analysis of variance, using the Sisvar software[19] and, when significant differences were observed by the F test, regression analysis was used to evaluate irrigation levels and Skott-Knott mean test for comparison between seedling types. In case of interaction between the factors, the irrigation factor was evaluated within each type of seedling.

## RESULTS AND DISCUSSION

An interaction was verified between the factors types of seedlings and irrigation levels, at 5% probability by the F test, for the variables transpiration and thickness of the palisade parenchyma. The other variables, when significant differences were found, were analyzed separately according to the treatments.

Plants obtained through cuttings showed greater photosynthetic activity than those obtained through somatic embryogenesis after the period in which they were submitted to the proposed water regimes (Table 3).

Even with the superiority of the photosynthetic activity of the plants obtained through cutting (Table 3), the data allowed the inference that, as the soil moisture increased, the photosynthetic activity raised in both types of seedlings (Figure 1).

Photosynthetic activity depends on water availability, light and temperature conditions and, with greater availability of water in the soil, and consequent greater hydration of tissues, as well as greater absorption of nutrients, it was observed that higher values of photosynthetic activity were found in both types of seedlings (Figure 1). In this way, the small availability of water in the soil associated with a possible greater transpiration of the plants, limited the photosynthetic rate, particularly of the seedlings produced through somatic embryogenesis, which can have a negative effect on the initial development of the crop.

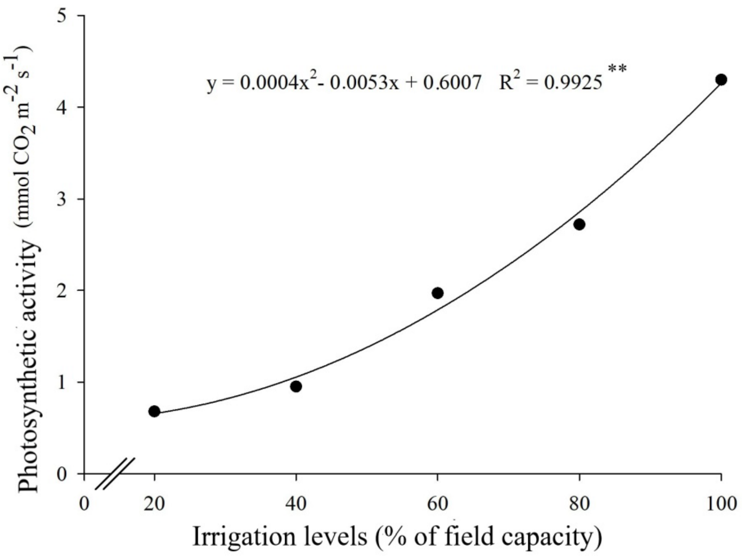
Similar to the photosynthetic activity, the development of the palisade parenchyma was greater in plants produced through cuttings (Table 4), with an increase in its thickness also occurring with the increase in the irrigation levels (Figure 2).

It can be seen in Figure 2 that the plants obtained through cuttings presented maximum thickness of the palisade parenchyma when they were kept in soil with 78% of available water in the soil, and from that point onwards, they resumed their smaller thicknesses of the palisade

**Table 3:** Photosynthetic activity of plants produced through cuttings and somatic embryogenesis during crop implantation

| Seedling Type | Photosynthetic activity (µmol $CO_2$ $m^{-2}$ $s^{-1}$) |
|---|---|
| Cutting | 2.38 a* |
| Somatic embryogenesis | 1.87 b |
| Coefficient of Variation (%) | 29.53 |

*Means followed by different letters show diferences by the test of Skott-Knott, at 5% of significance.



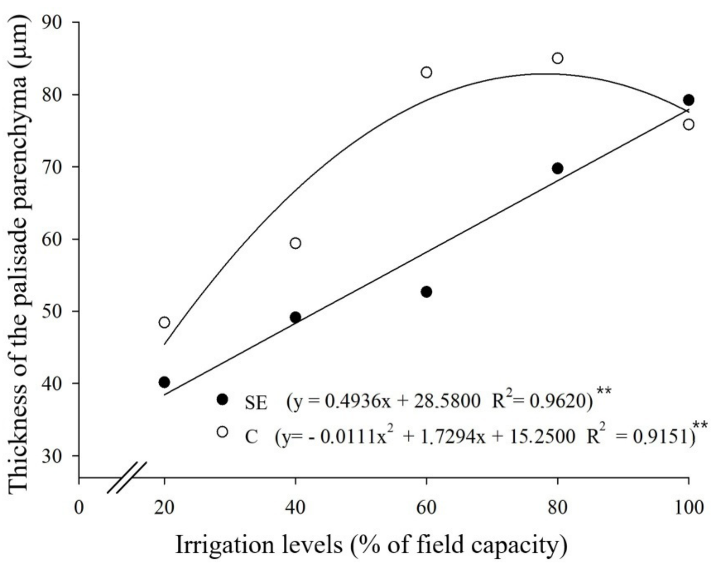

**: Significant at 1% probability level

**Figure 1**: Photosynthetic activity according to the percentage of water available in the soil.

**Table 4:** Palisade parenchyma thickness in plants produced through cuttings and somatic embryogenesis

| Seedling type | Palisade Parenchyma Thickness (µm) |
|---|---|
| Cutting | 70.3 a* |
| Somatic embryogenesis | 58.2 b |
| Coefficient of Variation (%) | 15.25 |

*Means followed by different letters show differences by the test of Skott-Knott, at 5% of significance.




**: Significant at 1% probability level

**Figure 2**: Thickness of the palisade parenchyma of plants obtained through somatic embryogenesis (SE) and cuttings (C), according to the percentage of water available in the soil.

parenchyma. The seedlings obtained through somatic embryogenesis showed a linear increase in the thickness of the palisade parenchyma, with an increase in soil moisture up to 100% of the available water, but with lower values than those obtained by cuttings in the various water conditions tested. This finding suggests greater plasticity (adaptation) of seedlings produced through cuttings in relation to those produced through somatic embryogenesis, as well as the thickness of the palisade parenchyma, as in the results obtained with photosynthetic activity (Table 3 and Figure 1).

According to Coelho *et al*.[20], the palisade parenchyma is the tissue with the greatest specialization for $CO_2$ fixation. Therefore, its greater development can provide greater efficiency in photosynthetic activity. Thus, higher values of photosynthetic activity are linked to greater development of the palisade parenchyma.

Oliveira *et al*.[14] also found that plants subjected to greater soil water availability had greater palisade parenchyma thickness, and in other cases, it was possible to observe that coffee trees with better performances under water stress conditions also developed better palisade parenchyma, to the detriment of the spongy one as a way for some genotypes to adapt to water deficit conditions[21].

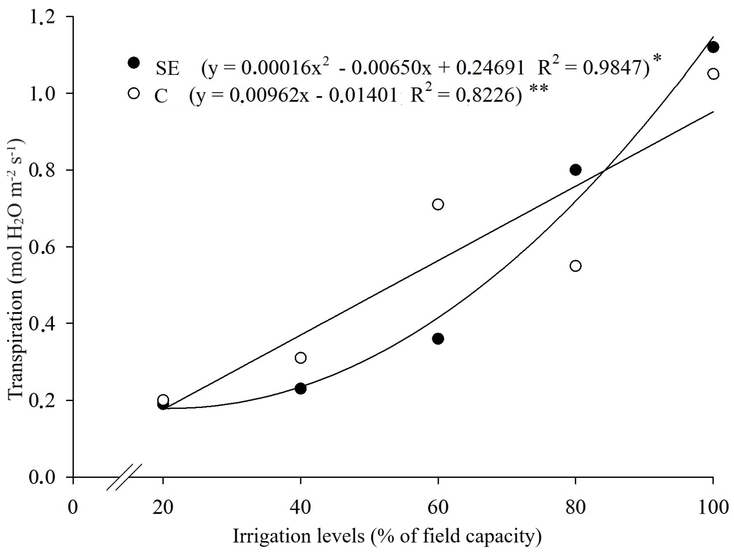
As well as the relationship between palisade parenchyma thickness and photosynthetic activity, different soil moisture levels also influenced transpiration. The results obtained show that both plants produced through cuttings and through somatic embryogenesis tend to increase transpiration with increasing soil moisture (Figure 3).

Plants with greater availability of water in the soil naturally promote greater losses through transpiration[22], which is a key process for nutrient absorption. As soil water availability is reduced, transpiration rate values decrease, as a result of stomatal closure.[23] This defense mechanism against water deficit, is important for plants to reduce greater water losses.[24]

It is also observed that when plants are under some type of stress (in this case, water deficit), they reduce stomatal conductance, also decreasing the rate of photosynthesis under these conditions[8], a fact that was observed in this works in the two types of assessed seedlings, in a similar way, that is, without interaction between the factors. (Figure 4)

The relationship between transpiration and stomatal conductance factors has already been reported in other works, where stomatal regulation helps balance water relations in the plant and soil.[25] Stomatal conductance is directly related to the amount of water available to the plant, and the greater this amount, the greater the conductance, allowing full plant growth.[26]

The first indicator of water deficiency in the coffee plant is the reduction in the stomatal conductance, which is a very important factor in the evaluation of possible water stress faced by the crop.[8] Such reduction was also observed by Santos et al[21] who also worked with the coffee crop.




*: Significant at 5% probability level.
**: Significant at 1% probability level.

**Figure 3**: Transpiration of plants obtained through somatic embryogenesis (SE) and cuttings (C) according to the percentage of water available in the soil.

In addition to the reduction of stomatal conductance in coffee plants under water deficit, Peloso et al.[27] also found in coffee plants, a reduction in net $CO_2$ assimilation, and in transpiration rates, in addition to a lower internal concentration of $CO_2$, since these factors are interconnected in conditions of low values of available water in the soil.

It was also found in the present work a reduction in stomatal density as soil water availability increased in the two types of assessed seedlings (Figure 5). That is, under conditions of water deficit, the plant increased stomatal density as a form of resilience/adaptation to this condition.

It could be seen through the comparison among the results of the present study for stomatal density between the two types of seedlings evaluated that the plants originating from seedlings though somatic embryogenesis adapted with a greater number of stomata per $mm^2$ (Table 5) in the conditions of water deficit, so it could be inferred that there was a greater adaptation of this type of seedlings in relation to those produced by cuttings.

Thus, the anatomical adaptations that occur in coffee plants in conditions of a poor amount of water available in the soil, allow greater resistance to dehydration and

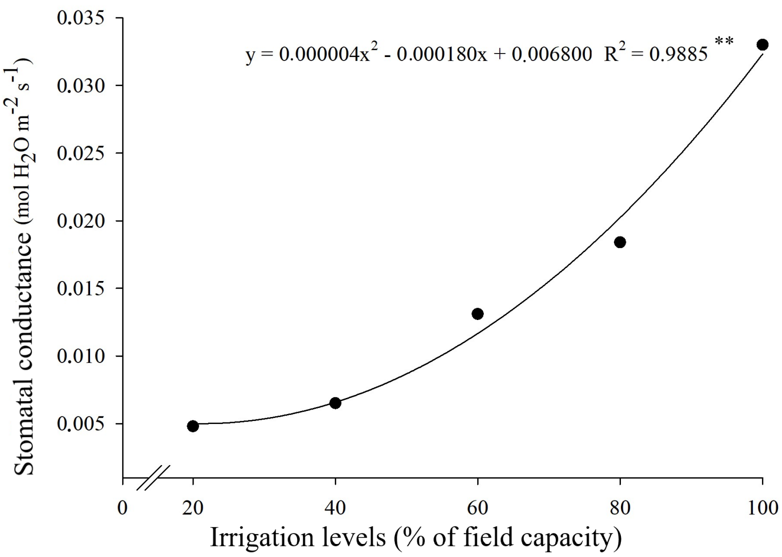



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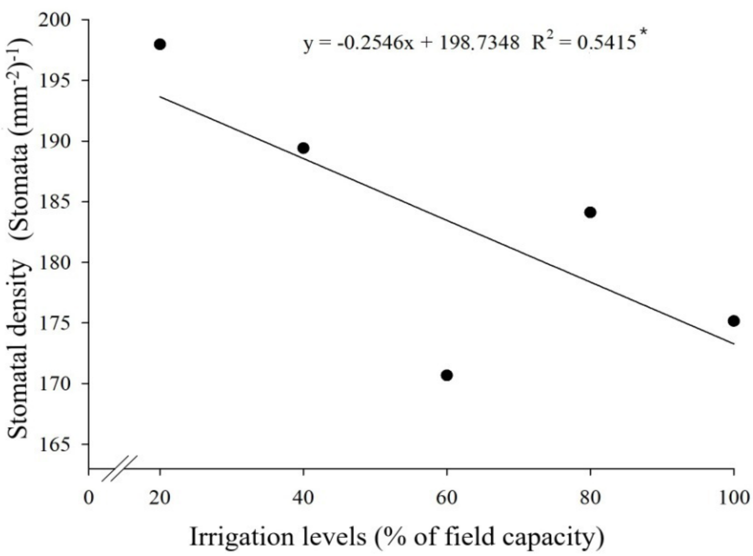
**Figure 4**: Stomatal conductance according to the percentage of water available in the soil.




*: Significant at 5% probability level.

**Figure 5**: Stomatal density of leaves according to the percentage of water available in the soil.

optimize the transport of solutes in the plant(28,29), similar to adaptation observed also in the stomatal density evaluations.

The increase in the flow of $CO_2$ improve photosynthetic efficiency and photo assimilate production(30), being a form of compensation for water limitations, resulting in a relationship between the factors stomatal density and water deficit.

Therefore, the functioning of the stomata is directly linked to the physiology of the plants, since, when open, they allow the assimilation of $CO_2$ and the loss of water. On the other hand, stomatal closure reduces the entry of $CO_2$ into the rubisco carboxylation sites inside the chloroplasts, maintaining a greater amount of water in the tissues, thus reducing the risk of dehydration(31), and in turn, reducing photosynthesis, a fact which was observed in this work.

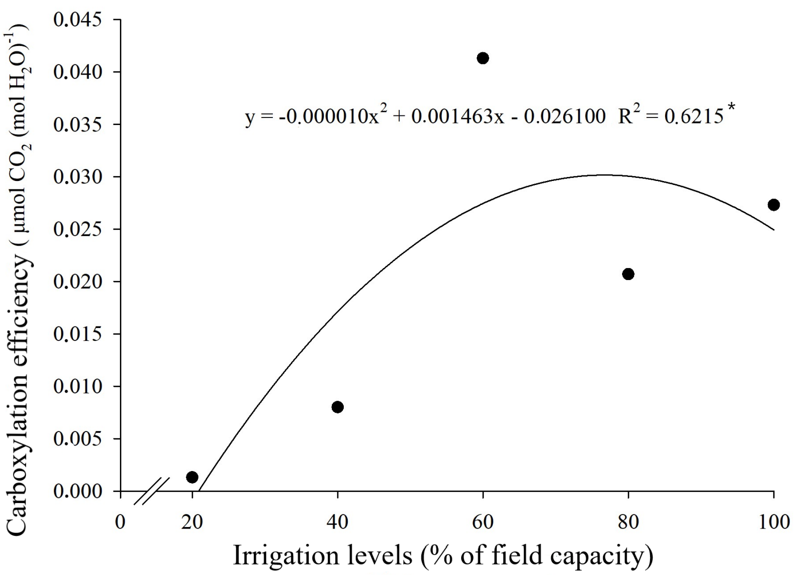
For a better understanding of the $CO_2$ assimilation process, this work evaluated the instantaneous efficiency of carboxylation, which reached the maximum point of the equation found with 78% of water available in the soil (Figure 6), for both types of assessed seedlings. That is, under conditions of water deficit, coffee plants (both from cuttings and from somatic embryogenesis) reduce the $CO_2$ assimilation.

Other works have also shown a reduction in carboxylation activity, in periods of water shortage in the soil (32,33) as the restriction of water supply to the plant impairs the full functioning of its physiological system, leading to a reduction in the $CO_2$ fixation and consequently, the production of energy. In addition, the reduction in stomatal conductance under conditions of water shortage is associated with a reduction in the carboxylation efficiency values as greater $CO_2$ inputs into the leaves, which is reflected by stomatal conductance values, allow for greater carboxylation activities, or that is, $CO_2$ assimilation. All these factors explain the higher values of photosynthetic activity found in conditions of higher soil moisture.

**Table 5:** Stomatal density in seedlings produced through cuttings and somatic embryogenesis

| **Seedling type** | **Stomatal density (stomata $mm^{2\ -1}$)** |
|---|---|
| Cutting | 171.9 b* |
| Somatic embryogenesis | 195.0 a |
| Coefficientof Variation (%) | 10.58 |

*Means followed by different letters show differences by the test of Skott-Knott, at 5% of significance.




*: Significant at 5% probability level

**Figure 6**: Carboxylation efficiency according to the percentage of water available in the soil.

## CONCLUSIONS

Both plants derived from cuttings and somatic embryogenesis are resilient to water deficit in the phase corresponding to crop implantation.

Plants from seedlings through cuttings reach higher values of photosynthetic activity and thickness of the palisade parenchyma in the absence of water deficit in relation to plants from seedlings through somatic embryogenesis, showing greater growth potential in the crop implantation phase, without water limitation.

In general, coffee plants from seedlings produced in somatic embryogenesis have higher stomatal density than those produced through cuttings.

Under water deficit conditions, seedlings produced by somatic embryogenesis and cuttings showed a reduction in transpiration and stomatal conductance, as well as an increase in stomatal density.

## ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

The authors would like to thank the Federal Institute of Education, Science and Technology of Espírito Santo (IFES) and the National Council for Scientific and Technological Development (CNPq) for their financial support. The authors declare that there is no conflict of interest in conducting and publishing the work.

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