

# ANATOMIC AND PHYSIOLOGICAL MODIFICATIONS IN SEEDLINGS OF *Coffea arabica* CULTIVAR SIRIEMA UNDER DROUGHT CONDITIONS

## Modificações anatômicas e fisiológicas em mudas de *Coffea arabica* cultivar Siriema submetidas ao déficit hídrico

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### ABSTRACT

Due to the weather changes prognostic for the coming years, the understanding of water deficit and physiological responses of plants to drought becomes an important requirement in order to develop technologies such as mechanisms to assist plants to cope with longer drought periods, which will be essential to maintenance of Brazilian and worldwide production. This study aimed to evaluate ecophysiological and anatomical aspects as well as the nitrate reductase activity in Siriema coffee seedlings subjected to four treatments: Daily irrigated, non-irrigated, re-irrigated 24 hours and re-irrigated 48 hours after different stress periods. Non-irrigation promoted a reduction in leaf water potential being accentuated from the ninth day of evaluation onwards. Re-irrigation promoted a partial recovery of the plant water potential. Non-irrigated plants showed an increase in stomatal resistance and reduction of transpiration and nitrate reductase activity. In the roots, there was a decrease in nitrate reductase activity under water stress. Leaf anatomical modifications were significant only for the adaxial surface epidermis and palisade parenchyma thickness, this latter characteristic being higher in control plants. Stomatal density and polar and equatorial diameter ratios showed the highest values in plants under water stress. In the roots, differences only in the cortex thickness being bigger in the non-irrigated treatment could be observed. Therefore, Siriema coffee plants under water stress show physiological, biochemical and anatomical modifications that contribute to the tolerance of this genotype to these conditions.

**Index terms:** Coffee tree, ecophysiology, water stress, nitrate reductase.

### RESUMO

Em razão do prognóstico de mudanças climáticas nos próximos anos, a compreensão do déficit hídrico e das respostas fisiológicas de plantas ao mesmo, torna-se uma importante condição para o desenvolvimento de tecnologias, como mecanismos que auxiliem as plantas a suportar períodos mais longos de seca, o que será essencial para a manutenção da produção mundial e brasileira. Neste estudo, objetivou-se avaliar aspectos ecofisiológicos e anatômicos além da atividade da redutase do nitrato em mudas de café Siriema submetidas a quatro tratamentos: irrigadas diariamente, não irrigadas, re-irrigadas 24 horas e re-irrigadas 48 horas após os diferentes períodos de estresse. A ausência de irrigação promoveu redução no potencial hídrico foliar, sendo mais acentuada a partir do nono dia de avaliação. A re-irrigação das mudas promoveu uma recuperação parcial do estado hídrico das plantas. Nas plantas não irrigadas observou-se um aumento na resistência estomatal e diminuição da transpiração, reduzindo a atividade da redutase do nitrato nas folhas. Nas raízes, houve um declínio na redutase do nitrato sob estresse hídrico. Ocorreram modificações na anatomia foliar apenas na espessura da epiderme da face adaxial e parenquimata paliádica, sendo maior nas plantas controle. A densidade estomatal e razão diâmetro polar e equatorial demonstraram maiores valores nas plantas sob déficit hídrico. Nas raízes, foi observada diferença apenas na espessura do córtex, sendo esta maior no tratamento não irrigado. Dessa forma, o café Siriema sob condições de estresse hídrico apresenta modificações fisiológicas, bioquímicas e anatômicas que contribuem para a tolerância desse genótipo nessas condições.

**Termos para indexação:** Cafeeiro, ecofisiologia, estresse hídrico, redutase do nitrato.

### INTRODUCTION

Brazil is the main world producer of coffee, which after oil, is the commodity most commercialized worldwide (Da Matta; Ramalho, 2006). Since the beginning of coffee production in Brazil, it has been directly related to economic development and industrialization, therefore

it is one of the most important cultures in the country. In 2012, the production was estimated at 50.48 million bags of 60 kg, which represents a 16.1% raise compared to the production in 2011. (Companhia Nacional De Abastecimento - CONAB, 2012)

Coffee tree flowering is induced by rainfall or irrigation after a period of water deficit (Carr, 2001).

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Nevertheless, longer periods of drought are becoming more common and can severely affect plant growth and production yield (Arruda; Grande, 2003). A physiological response sensitive to the water potential decrease is the modification in protein synthesis and, due to that, enzymes such as Nitrate Reductase (NR) are strongly inhibited (Marur; Mazzafera; Magalhães, 2000). The NR has been used in the evaluation of modifications related to environmental factors, for instance: radiation, hydric status and nitrogen availability (Marur; Mazzafera; Magalhães, 2000; Carelli; Fahl, 2006; Lima et al., 2008).

The water deficit can be related to significant restrictions in carbon assimilation by plants, and adaptation of tolerant plants to such conditions involve physiological and anatomical modifications (Maseda; Fernández, 2006).

Several investigations have been conducted to study water stress tolerance in *Coffea canephora* (Lima et al., 2002; Pinheiro et al., 2005; Praxedes et al., 2006; Silva et al., 2010) and *C. arabica* (Cai et al., 2005; Dias et al., 2007; Batista et al., 2010). The latter study demonstrated potential differences among 15 genotypes of *C. arabica* for drought tolerance, which confers higher leaf water potential and anatomic structure specialization of leaves to plants with higher potential to be tolerant. Leaf anatomy modifications such as mesophyll and epidermis thickening, increase of stomata density and functionality were demonstrated as potentially related to tolerance to different environmental stresses, such as drought and flooding (Grisi et al., 2008; Souza et al., 2010). Modifications in the vascular system and cortex thickness of roots in plants cultivated under stress can be observed, and can be associated with enzymatic activity variations (Pereira et al., 2008; Souza et al., 2009; Pereira et al., 2010).

Nevertheless, although progress has been made indicating the Siriema as drought tolerant (Dias et al., 2007; Grisi et al., 2008), it is necessary to understand the structure and physiological modifications in tolerant genotypes under different water stress conditions. The understanding of water deficit and physiological responses of plants to drought becomes an important requirement in order to develop technologies such as mechanisms to assist plants to cope with longer drought periods, which will be essential to maintenance of Brazilian and worldwide production.

Considering that drought is one of the main constraints to the coffee production, this work aimed at evaluating physiological, anatomical and enzymatic

(Nitrate Reductase) modifications in Siriema seedlings under different drought conditions.

## MATERIAL AND METHODS

### Plant material and growing conditions

The experiment was conducted in a greenhouse with a 50% light reduction cover. This greenhouse was located in the Plant Physiology experimental area of the Federal University of Lavras, Minas Gerais, Brazil. Six-month-old *Coffea arabica* L. Siriema seedlings were used for the experiments. The plants were grown in plastic bags (three liter capacity).

The plants were daily irrigated, and the water potential was kept at field capacity for three weeks. When the treatments started, one group of seedlings was continuously irrigated (control) while another group was not irrigated for up to 30 days period (Non-irrigated). In addition, in every 3 days, a subgroup of plants subjected to drought conditions was re-irrigated and evaluated 24 and 48 hours later (Re-irrigated). Evaluations and harvesting of roots and leaves were performed every 3 days for a 30 day period. All the plants were grown in a completely randomized design, with four repetitions per evaluation period. The biological repetition consisted of a single plant.

### Physiological measurements

The leaf water potential ( $\Psi_w$ ) was evaluated with a pressure chamber (Soil moisture – Mod. 3005), early in the morning, in the fourth pair of fully expanded leaves counted from the apex of the plagiotropic branch, in four seedlings per treatment, every three days until the end of the experiment.

The stomata resistance and transpiration were evaluated between 10 AM and 11 AM, by using a porometer (*Steady State Porometer*, Licor 1600M). For measurement, a fully expanded leaf of the third node counted from the apex of the plagiotropic branch was used, in four seedlings per treatment.

### Nitrate Reductase (NR) activity

The *in vivo* activity of the Nitrate Reductase enzyme was determined in roots and leaves according to Queiroz et al. (1993) methodology with modifications. The harvesting of fully expanded leaves in the middle region of the canopy and whole roots was performed at 9 A.M. and 2 P.M., respectively, and the samples were immediately placed on ice and transferred to the laboratory. Leaf discs of 3.5 mm diameter weighing approximately 400 mg of fresh weight were taken with the aid of a cork borer and transferred

to incubation media composed of 200 mM Phosphate Buffer, pH 7.5, 100% propanol, Triton X-100, 1000 ppm chloramphenicol. Furthermore, 0.5 mL of Potassium Nitrate (1M) was added to each flask and these were subjected to vacuum for 2 minutes and then transferred to agitated water bath at 30 °C for 40 minutes in the dark.

For the harvested roots, the samples were washed with distilled water and fragmented with a blade. Furthermore, the samples were dried on paper towel and 400 mg of root material were incubated in 4.5 mL of the same buffer described above, except for the Triton X-100. The flasks were subjected to bubbling with nitrogen gas for 2 minutes and then 0.5 mL of Potassium Nitrate (1M) were added to each sample and the flasks incubated in water bath at 30 °C for 40 minutes in the dark.

For quantification of the formed nitrite, aliquots from incubation media were taken and mixed with the reaction media, composed by 1 mL of 1% sulfanilamide in 1.5N HCl, 1 mL of 0.02% N-1-naphthyl-ethylenodiamine and 1.5 mL of distilled water. The absorbance reading was performed in a spectrophotometer at 540 nm.

### Anatomical Evaluation

Thirty days after suspension of irrigation, entire roots were harvested as well as completely expanded leaves on the second node counted from the apex of the orthotropic branch in the crown median region of four individuals for each treatment, corresponding to the repetitions. The material was fixed and stored in ethanol 70%.

A table microtome type LPC was used to obtain the cross sections in the middle third of leaves and roots. The paradermal cuts were performed manually in the middle third of the abaxial surface of leaves. For clarification of the cross and paradermic sections 5% sodium hypochlorite was used and, for staining, a mixture of safranin and astra blue was used for of the transverse sections (Kraus; Arduin, 1997) and the paradermic sections were stained with 1% safranin solution. 50% glycerol was used in the slide mounts.

In leaves, the thickness of the adaxial and abaxial epidermis, palisade and spongy parenchyma and of the leaf limbus, were measured, while in the roots, the thickness of the epidermis and cortex were measured. Five fields per repetition were observed, totaling 20 fields per treatment. The stomatal density was expressed as number of stomata per mm<sup>2</sup> (Melo et al. 2007). Five fields of four individuals per treatment to determine the stomatal density on abaxial epidermis and measurement of the polar and equatorial stomata diameters were used.

The measurements were performed using the software SigmaScan Pro 5 and the data subjected to

statistical analysis of anatomy were evaluated with the aid of the software Sisvar (Ferreira, 2000) with Tukey test at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Leaf water potential

For the control group, the leaf water potential presented minimal changes during the experiment period, with values of about -0,2 MPa (Figure 1). However, for the seedlings submitted to the suspension of irrigation, a linear decrease was observed in the water potential from the ninth day of the experiment reaching -3.5 MPa at 30 days. For the seedlings that were re-irrigated, the evaluation conducted after 24 hours showed an expressive recovery of the water potential, however, at 27 days, the water potential still presented values under -2.0 MPa. For the treatment 48 hours after re-irrigation the highest water potential recovery was observed, arriving at values close to those observed in the control at 24 days. At 30 days, this evaluation was not conducted in the treatments 24 and 48 hours after re-irrigation, due to the leaf abscission occurred in these plants.

At 27 days the water potential was still close to -2.0 MPa, values considered harmful to the coffee plant, because they cause reduction of stomatal conductance and, consequently, the photosynthesis rates (Lima et al., 2002; Batista et al., 2010). These results show that ‘Siriema’ coffee plant seedlings survive prolonged periods of water deficit and present a fast recovery of their hydric state when newly re-irrigated. Similar results were observed by Pinheiro et al. (2005) after re-irrigating *Coffea robusta* clones submitted to the hydric stress, emphasizing the role of the “hydric status” of the leaf on stomatal control.

The leaf water potential describes its energy state, whose gradients explain the water flow in the soil-plant-atmosphere system. This characteristic can be used as an indicator of more drought tolerant coffee plants (Batista et al., 2010). According to Rena and Maestri (2000), in coffee plants in the field, the predawn leaf water potential rarely falls to values under -1.5 MPa, because under natural conditions the water deficit is established slowly and gradually, allowing the plants to adjust to that condition, minimizing environmental tension impacts.

The stomatal resistance for the plants maintained irrigated underwent little alteration during the experimental period (Figure 2). However, the suspension of irrigation resulted in expressive increases in the stomatal resistance from 12 days after the beginning of the experiment arriving at approximately 325 s cm<sup>-1</sup> at 30 days. Even the seedlings

submitted to re-irrigation demonstrated an increase in the stomatal resistance, but with lower values than those observed for the non-irrigated plants. As such, the stomatal resistance had behavior inversely proportional to the water potential (Figures 1 and 2).

The stomatal resistance is simultaneously affected by various environmental factors (Silva et al., 1998), however, the stomatal closing avoids the occurrence of damage to the metabolic systems, adjusting them to the moisture deficit in the plant, resulting in the temporary maintenance of the water potential in the leaf.

As the stomatal resistance increased (Figure 2) the transpiration decreased gradually (Figure 3). In the control, a lower stomatal resistance was observed and consequently, these plants showed higher transpiration. On the other hand, the suspension of the irrigation during the experiment

promoted a drastic reduction of the transpiration, presenting values close to zero at the end of the appraised period. For the re-irrigated plants there was similar behavior for the two different evaluation periods (24 and 48 h) during the experimental period, where only at 12 days was there an expressive recovery of the transpiration.

One of the first responses used by the coffee plant to minimize water loss occurred with transpiration under hydric stress conditions is the closing of the stomata (Batista et al. 2010). In adaptation studies of cultures to low precipitations areas, the main objective is the increase of productivity associated to rational and economical water use, however, according to Maseda and Fernández (2006), there is the risk of complete exhaustion of the water available in the soil, with serious structural and physiological damage to the tissues.

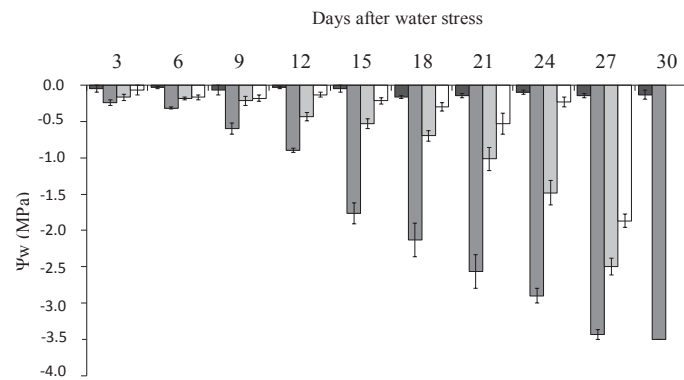


Figure 1 – Leaf water potential of Siriema coffee seedlings submitted to water deficit. Irrigated plants (■), non-irrigated (■), 24 hours after re-irrigation (■) and 48 hours after re-irrigation (□). Bars represents mean standard error of 4 repetitions.

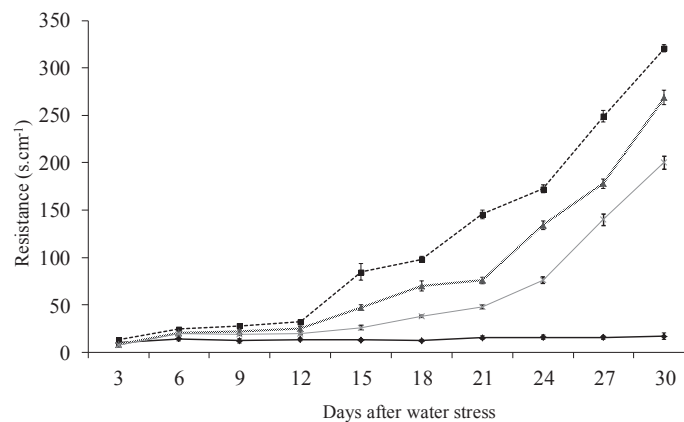


Figure 2 – Stomatal resistance for leaves of Siriema coffee seedlings submitted to water deficit. Irrigated plants (◆), non-irrigated (---■---), 24 hours after re-irrigation (▲) and 48 hours after re-irrigation (×). Bars represent mean standard error of 4 repetitions.

The transpiration rate, variable according to the different hydric regimes, shows that the plant can activate water loss defense mechanisms when soil moisture is deficient. Such procedure can be harmful to full growth and vegetable development, but it guarantees survival, as long as the water deficit is not highly severe and lingering (Silva et al. 1998).

Nitrate reductase (NR) appraised in the leaves of the control plant, in general, underwent little variation in its activity during the experimental period (Figure 4). However, for the plants submitted to water deficit, there was a quick decline in the NR activity from the first to the 12th day of evaluation in relation to the control. Starting from this period, a linear decline was observed until the last evaluation conducted at 24 days for this treatment,

a fall of approximately 65% between the beginning and the end of the evaluations. This expressive reduction in the NR activity in the leaves coincides with the period of higher water potential decline (Figure 1), with values under  $-2.0$  MPa around the 18th day of evaluation, considered critical for the coffee culture. However, the re-irrigated treatments, showed an NR activity recovery in relation to the plants submitted to hydric stress starting from the 12th day, the highest difference being observed on the 15th day for the 48 hours of re-irrigation treatment, returning to an activity similar to the control. For the suspension of irrigation and the re-irrigated treatments, the nitrate reductase showed activity only until the evaluation conducted on the 24th day after the start of the experiment.

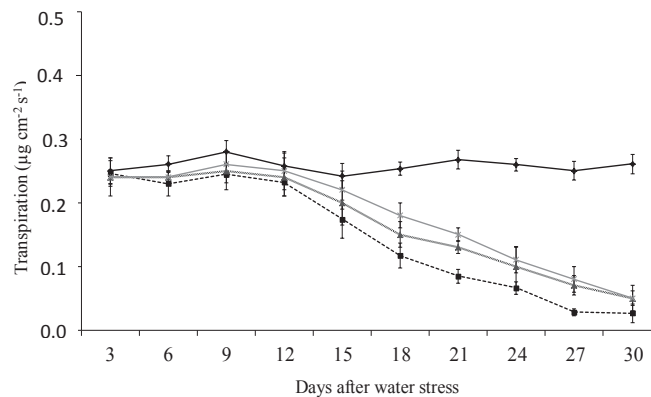


Figure 3 – Transpiration for leaves of Siriema coffee seedlings submitted to water deficit. Irrigated plants (◆), non-irrigated (◻), 24 hours after re-irrigation (▲) and 48 hours after re-irrigation (×). Bars represent mean standard error of 4 repetitions.

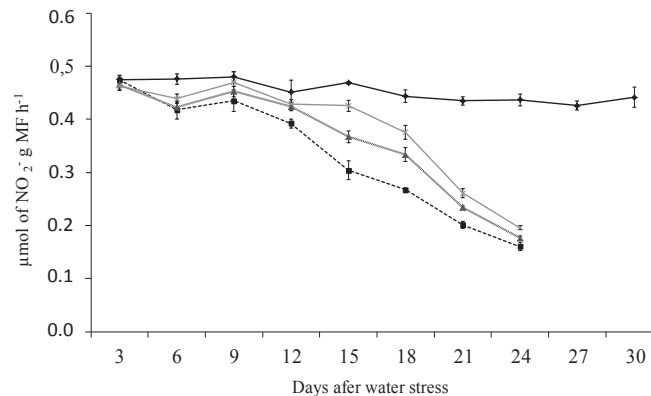


Figure 4 – Nitrate Reductase activity for leaves of Siriema coffee seedlings submitted to water deficit. Irrigated plants (◆), non-irrigated (◻), 24 hours after re-irrigation (▲) and 48 hours after re-irrigation (×). Bars represent mean standard error of 4 repetitions.



In the roots, there was a decline in the NR activity in the control plants starting from the ninth day of evaluation, this fact can be related to the limited space for root development, besides a decrease in the amount of the nitrate present in the substratum (Figure 5). This same behavior was observed in the non-irrigated treatment, in other words, over time the NR activity declined, however, to values significantly inferior to those observed in the control. The re-irrigation of the plants was only efficient for the recovery of the NR activity in the roots in the periods between days 12 to 24, being more expressive on the 15th and 18th day for the 48 hour treatment.

The nitrate reductase activity is highly modulated by environmental factors, such as: radiation, temperature, nitrate and water (Freitas et al., 2007; Carelli; Fahl, 2006). The presence of nitrate in the soil promotes stimulation to *de novo* synthesis of the referred enzyme, however if the plant undergoes a situation of hydric stress its activity can be reduced (Marur; Mazzafera; Magalhães, 2000). The availability of ( $\text{NO}_3^-$ ) is one of the main factors that can modify the NR activity (Lima et al., 2008). Thus, the decrease observed in the NR activity can be related to a decrease in the flow of ( $\text{NO}_3^-$ ) from the substratum to the roots due to lack of moisture.

For the characteristics related to the leaf anatomy (Table 1), significant differences occurred in the thickness of the epidermis of the adaxial face and in the palisade parenchyma, the highest values being observed in the control plants, with averages 12.50% superiors for the epidermis of the adaxial face and 8.59% for the palisade

parenchyma. The stomatal density was 42.42% superior in the plants under hydric stress, and they even presented values 19.72% superior in the ratio between the polar and equatorial diameters in relation to the control. That characteristic can serve as a plant response indicator as to its hydric state. In the present case, the highest ratio was observed in the plants under stress, showing that their stomata had a more elliptic morphology in relation to the plants maintained irrigated, a mechanism adopted by the plants to keep the water present in its interior at a maximum. Grisi et al (2008) verified that the cultivar Siriema under hydric stress conditions can promote stomata development with a higher polar/equatorial diameter ratio the in relation to less drought tolerant cultivars. Other environmental stresses can also promote an increase in that ratio in function of a better response to foliar gas exchanges (Souza et al., 2010).

Modifications in the density and size of the stomata are quite variable characteristics in function of the environment and the decrease in the stomata size is an event recognized as important in gas exchange regulation in plants under stress (Castro et al., 2005; Souza et al. 2010). Therefore, the increase in the stomatal density is an alternative to the appropriate supply of  $\text{CO}_2$  necessary for the photosynthesis, without there being excessive water loss to the detriment of the stomata with smaller sized pores (Melo et al. 2007). These results are in agreement with several authors that observed that the number of stomata related to photosynthesis maintenance, can vary in plants growing under different environmental conditions (Souza et al., 2010; Batista et al., 2010).

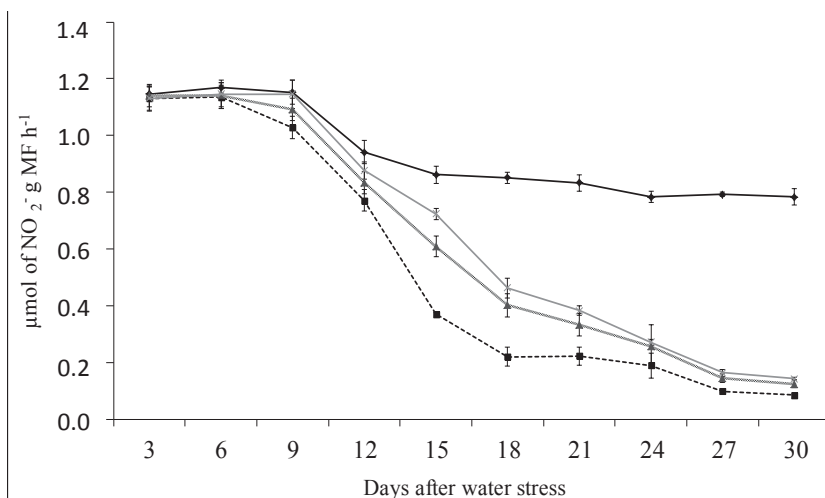


Figure 5 – Nitrate Reductase activity for roots of Siriema coffee seedlings submitted to water deficit. Irrigated plants (♦), non-irrigated (--■--), 24 hours after re-irrigation (▲) and 48 hours after re-irrigation (×). Bars represent mean standard error of 4 repetitions.

Table 1 – Stomatal density and thickness of cells and tissues of Siriema coffee plants submitted to water deficit.

Evaluated Characteristics	Treatment			
	Control		Water Deficit	
	Leaf	Roots	Leaf	Root
Adaxial epidermis thickness ( $\mu\text{m}$ )	027.83 a	-	024.35 b	-
Abaxial epidermis thickness ( $\mu\text{m}$ )	024.41 a	-	022.53 a	-
Thickness of palisade parenchyma ( $\mu\text{m}$ )	069.33 a	-	063.37 b	-
Thickness of spongy parenchyma ( $\mu\text{m}$ )	175.10 a	-	167.01 a	-
Leaf thickness ( $\mu\text{m}$ )	281.61 a	-	264.82 a	-
Stomatal density ( $\text{n}^\circ/\text{mm}^2$ )	123.02 b	-	175.21 a	-
Polar diameter ( $\mu\text{m}$ )	024.78 b	-	027.36 a	-
Equatorial diameter ( $\mu\text{m}$ )	016.92 a	-	015.60 b	-
Polar/Equatorial diameter ratio ( $\mu\text{m}$ )	001.47 b	-	001.76 a	-
Epidermis thickness ( $\mu\text{m}$ )	-	022.15 a	-	023.45 a
Cortex thickness ( $\mu\text{m}$ )	-	158.74 b	-	197.04 a

\*Means followed by the same letter, among treatments and within the same organ, do not differ by Tukey test ( $p < 0.05$ ).

In the roots, there was significant difference only in the thickness of the cortex, where the largest values were observed for the plants under hydric deficiency. The thickness of the cortex is a characteristic that can be related to the hydraulic conductivity, since the lower the thickness of the cortex, the higher the hydraulic conductivity will be, being able to modify itself in function of the water availability in the environment (Pereira et al., 2008). That characteristic cannot only modify in function of the absence of water but also under other environmental stresses (Souza et al., 2009), the root being one of the main organs to respond to those conditions, for to anatomical modifications, as well as physiological (Pereira et al., 2010).

The greater thickness of the cortex, observed in the plants submitted to hydric stress, can be understood as a survival strategy under adverse water availability conditions (Silva; Castro; Soares, 2003). According to Pereira et al. (2008), the exodermis constitutes a mechanical protection for water flow control in the roots, having the restriction of the water reflux from the roots to the soil as principal function. The higher cortex thickness can be related to a higher water retention capacity in the roots, for allowing the cortical cells to store water in their protoplasts, thus contributing to the stress tolerance of this cultivar.

### CONCLUSIONS

The cultivar Siriema is capable of surviving periods of water deficiency, presenting metabolic,

physiological and anatomical modifications, and presents a fast recovery of their hydric state when newly re-irrigated.

The plants of the Siriema cultivar demonstrate transpiration restrictions when under hydric stress, promoted by the increase in stomatal resistance.

The nitrate reductase enzyme activity is reduced in the leaves and roots of the Siriema plants under hydric stress, reducing the assimilation of nitrogen.

The leaf anatomy Siriema under hydric stress demonstrates modifications in the stomata that allow better adaptation to those conditions. The roots of the Siriema cultivar demonstrate an increase in cortex thickness under hydric stress conditions increasing the root hydraulic retention capacity.

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### REFERENCES

ARRUDA, F.B.; GRANDE, M. A. Fator de resposta da produção do cafeeiro ao déficit hídrico em Campinas. *Bragantia*, 62(1):139-145, 2003.

- BATISTA, L.A. et al. Anatomia foliar e potencial hídrico na tolerância de cultivares de café ao estresse hídrico. **Revista Ciência Agronômica**, 41(3):475-481, 2010.
- CAI, Z.Q. et al. Responses of two field-grown coffee species to drought and re-hydration. **Photosynthetica**, 43(2):187-193, 2005.
- CARELLI, M.L.C.; FAHL, J.I. Partitioning of nitrate reductase activity in *Coffea arabica* L. and its relation to carbon assimilation under different irradiance regimes. **Brazilian Journal of Plant Physiology**, 18(3):397-406, 2006.
- CARR, M. K. V. The water relations and irrigation requirements of coffee. **Experimental Agriculture**, 37(1):1-36, 2001.
- CASTRO, E.M. et al. Aspectos anatômicos e fisiológicos de plantas de guaco submetidas a diferentes fotoperíodos. **Horticultura Brasileira**, 23(3):846-850, 2005.
- COMPANHIA NACIONAL DE ABASTECIMENTO-CONAB. **Safra 2012: terceira estimativa**. Brasília, 2012. Disponível em: < [http://www.conab.gov.br/OlalaCMS/uploads/arquivos/12\\_09\\_06\\_10\\_10\\_21\\_boletim\\_cafe\\_-\\_setembro\\_2012.pdf](http://www.conab.gov.br/OlalaCMS/uploads/arquivos/12_09_06_10_10_21_boletim_cafe_-_setembro_2012.pdf)>. Acesso em: 20/01/2013.
- DIAS, P.C. et al. Morphological and physiological responses of two coffee progenies to soil water availability. **Journal of Plant Physiology**, 164(12):1639-1647, 2007.
- FERREIRA, D. F. **SISVAR 4. 3: sistema de análise estatística**. Lavras: UFLA, 2000. Software.
- FREITAS, R.B. DE. et al. Adubação do cafeeiro com nitrato de potássio via solo e folha, no outono-inverno e primavera-verão: efeitos na atividade da redutase do nitrato, no crescimento das plantas e na produção. **Ciência e Agrotecnologia**, 31(4):945-952, 2007.
- GRISI, F.A. et al. Avaliações foliares em mudas de café 'Catucaí' e 'Sirriema' submetidas ao estresse hídrico. **Ciência e Agrotecnologia**, 32(6):1730-1736, 2008.
- KRAUS, J.E.; ARDUIM, M. **Manual básico de métodos em morfologia vegetal**. Seropédica: Edur. 1997. 221p.
- LIMA, A.L.S. et al. Photochemical responses and oxidative stress in two clones of *Coffea canephora* under water deficit conditions. **Environmental and Experimental Botany**, 47(3):239-247, 2002.
- LIMA, J.D. et al. Acúmulo de compostos nitrogenados e atividade da redutase do nitrato em alface produzida sob diferentes sistemas de cultivo. **Pesquisa Agropecuária Tropical**, 38(3):180-187, 2008.
- MARUR, C.J.; MAZZAFERA, P.; MAGALHÃES, A.C. Atividade da redutase do nitrato em algodoeiro submetido ao déficit hídrico e posterior recuperação da turgescência. **Scientia Agrícola**, 57(2):277-281, 2000.
- MASEDA, P.H.; FERNÁNDEZ, R.J. Stay wet or else: three ways in which plants can adjust hydraulically to their environment. **Journal of Experimental Botany**, 57(15):3963-3977, 2006.
- MATTA, F.M. DA; RAMALHO, J.D.C. Impacts of drought and temperature stress on coffee physiology and production: a review. **Brazilian Journal of Plant Physiology**, 18(1):55-81, 2006.
- MELO, H.C. et al. Alterações anatômicas e fisiológicas em *Setaria anceps* Stapf ex Massey e *Paspalum paniculatum* L. sob condições de déficit hídrico. **Hoehe**, 34(2):145-153, 2007.
- PEREIRA, F.J. et al. Evolução da anatomia radicular do milho 'Saracura' em ciclos de seleção sucessivos. **Pesquisa Agropecuária Brasileira**, 43(12):1649-1656, 2008.
- PEREIRA, F.J. et al. Atividade dos sistema antioxidante e desenvolvimento de aerênquima em raízes de milho 'Saracura'. **Pesquisa Agropecuária Brasileira**, 4(5):450-456, 2010.
- PINHEIRO, H.A. et al. Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. **Annals of Botany**, 96(1):101-108, 2005.
- PRAXEDES, S.C. et al. T. Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (*Coffea canephora* Pierre var. *kouillou*) leaves. **Environmental and Experimental Botany**, 56(3):263-273, 2006.



QUEIROZ, C.G.S. et al. Ritmo diurno na atividade da redutase de nitrato em folhas e raízes de *Coffea arabica* L. **Pesquisa Agropecuária Brasileira**, 28(7):787-795, 1993.

RENA, A.B.; MAESTRI, M. Relações hídricas no cafeeiro. **Revista Item**, 48:34-41, 2000.

SILVA, L.C. et al. Variação diurna da resistência estomática à difusão de vapor de água em amendoim irrigado. **Pesquisa Agropecuária Brasileira**, 33(3):269-276, 1998.

SILVA, S.; CASTRO, E.M.; SOARES, A. M. Efeito de diferentes regimes hídricos sobre características anatômicas de raiz de gramíneas promissoras para revegetação

ciliar de reservatórios hidrelétricos. **Ciência e Agrotecnologia**, 27(2):393-397, 2003.

SILVA, V. A. et al. Physiological response of Conilon coffee clone sensitive to drought grafted onto tolerant rootstock. **Pesquisa Agropecuaria Brasileira**, 45(5):457-464, 2010.

SOUZA, T.C. et al Morpho-anatomical characterization of root in recurrent selection cycles for flood tolerance of maize (*Zea mays* L.). **Plant Soil and Environment**, 55(11):504-510, 2009.

SOUZA, T.C. et al. Leaf plasticity in successive selection cycles of 'Saracura' maize in response to periodic soil flooding. **Pesquisa Agropecuária Brasileira**, 46(1):16-24, jan. 2010.