Comunicata Scientiae
Horticultural Journal

ORIGINAL ARTICLE
published: 12 June 2022
https://doi.org/10.14295/CS.v13.3590

Phenotypic plasticity of *Aloysia citrodora*: anatomical changes to water availability and seasons

Leonardo Antonio Thiesen[1]*, Marcos Vinícius Marques Pinheiro[2], Evandro Holz[3], Anderson Werner[3], Elder Eloy[3], Braulio Otomar Caron[3], Denise Schmidt[3]

[1]Federal University of Santa Maria, Santa Maria, Brazil
[2]State University of Maranhão, Brazil
[3]Federal University of Santa Maria, Frederico Westphalen, Brazil
*Corresponding author, e-mail: thiesen07@hotmail.com

Abstract

The objective was to evaluate the anatomical changes of the leaves of *Aloysia citrodora* submitted to different water availability during the seasons. The experiment was conducted in a greenhouse, in a randomized block design, bifactorial 4x4, with four seasonal factors (autumn, winter, spring, and summer) and simulations of water availability (25, 50, 75, and 100% of field capacity) with four repetitions. They were evaluated in µm: the thickness of the adaxial and abaxial cuticles, adaxial and abaxial epidermis, palisade and lacunous parenchyma, mesophyll, and leaf thickness of the transversal section and the mesophyll and thickness of the main rib and the length and width of the vascular system. In the autumn season, there was a reduction in the width of the vascular system and the thickness of the adaxial cuticle under greater water availability. In winter and under low water availability, there was a reduction in the thickness of the cuticle and adaxial epidermis, lacunous parenchyma, mesophyll and leaf thickness, mesophyll, and main rib thickness. In the spring with greater water availability increased in the adaxial and abaxial epidermis, mesophyll and thickness of the main rib, and length of the vascular system; and low water availability provided the greater thickness of the adaxial cuticle, palisade parenchyma, and leaf thickness. In the summer season with the greatest water availability, there was an increase in leaf thickness and adaxial cuticle and a reduction in palisade parenchyma, while low water availability increased the thickness of leaf mesophyll.

Keywords: drought stress, leaf anatomy, lemon verbena, seasonality

Introduction

The *Aloysia citrodora*, popularly known as lemon verbena, is a South American aromatic plant rich in essential oil with anesthetic and antibacterial properties, stimulating, antioxidant activities, among others (Bahramsoltani et al., 2018). Essential oil is a product of secondary metabolism that performs essential functions to physiological and ecological processes in the plant, mainly related to protection against stress, among them abiotic, which can be caused by temperature, water availability, light, nutritional deficiency (Isah, 2019), among others.

The water stress promotes morphological changes that result in reduced plant growth and development (Álvarez et al., 2011), being possible to occur during seasonal variations in the environment. To combat these stressful changes, the plants can acclimatize from the phenotypic plasticity, determined by the external and internal morphology, i.e. from the anatomical changes that occur in the leaves and other organs of the plant (Matesanz et al., 2010; Gratani, 2014).

The water deficiency can cause reduced growth due to the lower elongation of the stem (Litvin et al., 2016) and leaves, with a consequent reduction in total crop yield (Oz et al., 2015). Also, water availability directly influences leaf anatomy and plasticity in plant physiological responses (Du et al., 2019), and these changes are related to each species and plant production (Bisbis et al., 2018).

Changes in leaf structure determine the ability of plants to acclimatize to stressful environmental conditions (Schöttler & Tóth, 2014; Melo Júnior & Boeger, 2016), and generally, when subjected to a significant reduction in water availability, the leaves show a size reduction, in thickening of the cell wall (Tenhaken, 2015), thicker cuticle epidermis, thicker palisade tissue and greater ratio of palisade/lacunous parenchyma, in addition to

Received: 07 September 2020
Accepted: 24 January 2022

changes in sap-conducting tissues (Fang & Xiong, 2015). The characteristics and anatomical changes of leaf tissues can be considered as indicators of plant tolerance to different environmental conditions (Batista et al., 2010; Grisi et al., 2008), among them the loss of water to the environment (Vasellati et al., 2001; Makbul et al., 2011). Also, the anatomical characteristics of aromatic plants can provide characteristics capable of determining the best growing conditions, as well as demonstrating the plant's responses to environmental variations (Boeger et al., 2009; Martins et al., 2010).

Considering that plants have plasticity and promote anatomical changes in response to environmental changes, there are no reports in the literature about how *Aloysia citrodora* acclimatizes anatomically when they are subjected to adverse cultivation conditions. Therefore, the objective of the work was to evaluate the anatomical characteristics of leaves of *Aloysia citrodora* submitted to different water availability and seasonality.

Materials and methods

Plant material and cultivation conditions

The work was conducted in a greenhouse in the experimental area of the Federal University of Santa Maria, *Campus* Frederico Westphalen, located at 27°23'S, 53°25'O, and 493 m of altitude, from August 2015 to March 2017. According to the Köppen classification, the region's climate is of the Cfa type - humid temperate with hot summer, with maximum air temperatures in the warmer months above 22°C (Alvares et al., 2013). The protected environment was constituted by a galvanized steel structure (10 x 20 m and 3.0 m in height), covered with transparent low-density polyethylene film, 150 µm thick, treated against ultraviolet radiation, with 87% transmittance, being non-selective, arranged in the East-West direction.

The seedlings of *Aloysia citrodora* were vegetatively propagated in phenolic foam by the minicutting method, and for that, it consisted of removing small cuttings with three buds each (10 cm in length, approximately) from parent plants, being disinfected in sodium hypochlorite solution (1% active chlorine, for one minute), and later washing in distilled water and planted in phenolic foam. One cell of the phenolic foam was used for each minicutting, introducing a yolk in the substrate. The phenolic foam plates were maintained on a stand with constant sub-irrigation, in the form of a water slide, and the irrigation shifts were controlled using a timer with 15 minutes on and 60 minutes off. At night, there were only two periods of irrigation of 15 minutes. The water, after passing through the phenolic foam, returned to the reservoir.

After 13 days of irrigation with water, the use of a nutrient solution containing all macronutrients and micronutrients was started, with 25% of the normally used concentration: 0.5 g L^{-1} of Calcinit® (Composition: 15.5% N and 19% Ca); 0.4 g L^{-1} of Hidrogood Fert® (Composition: 9% N, 9% P_2O_5, 29% K_2O, 3% Mg, 3.9% S, 0.03% B, 0.01% Cu, 0.035% Mn, 0.02% Mo, 0.015% Zn and 0.007% Ni), and 0.03 g L^{-1} of chelated iron (Composition: 6% Fe), maintaining the electrical conductivity and pH of the solution at approximately 300 µS and 6.0, respectively. After 68 days, the minicuttings were transplanted to five-liter pots containing commercial substrate Carolina®, remaining for more than 84 days in these conditions.

Experimental conditions

To experiment, seedlings of 152 days were transplanted to pots with a capacity of 14 liters, filled with a thin layer of gravel (3 kg), and sieved soil mix (oxisol) + 10% tanned cattle manure. To avoid overheating and excessive water loss due to the evaporation of the soil, the pots were painted white on the outside, promoting greater reflection and less absorption of solar radiation.

The experiment was conducted in a randomized block design, bifactorial 4x4, with four levels of water availability (25, 50, 75, and 100% of field capacity) and four seasons (autumn, winter, spring, and summer), with four repetitions for each treatment and the experimental unit composed of one leaf per plant.

To standardize the effect of seasonality, the plants received water restriction from the period that corresponds to half of each season of the year, that is, 45 days of the corresponding season. Before the start of irrigation with different water availability, all plants were irrigated with 100% of the field capacity. Water management was based on the humidity of the pot, determined by the weighing method, based on the daily weighing of pots filled with soil, with the aid of a digital scale with a maximum capacity of 40 kg. The replacement of evapotranspiration water was performed whenever the variation between the initial mass of the vessel and the mass obtained on the day of the evaluation became equal to or greater than 2%. Therefore, the difference between the masses corresponded to the amount of water to be completed, if the water has a weight/volume ratio of 1:1. The temperature inside the greenhouse was monitored using thermometers of maximum and minimum temperature of the day. The average temperature was determined by the following equation: Average temperature (°C) = (maximum temperature + minimum temperature)/2.

Anatomical characterization of the leaves

At the end of each season, that is, approximately 45 days after the beginning of irrigations with different water availability, the morphometry of the internal leaf structure was evaluated using anatomical techniques. For this, one leaf per plant was collected at the fifth node of the apex towards the base. When the leaves were removed, the extremity of the leaves was cut (maintaining only the central region of the leaf), and immediately fixed in a 70% FAA solution, composed of formaldehyde (37%), ethanol (70%), and glacial acetic acid, remaining in a solution for 48 hours. Subsequently, the samples were dehydrated in an ethyl series and included in methacrylate (Historesin® - Leica Biosystems Nussloch, Heidelberg, Germany). To assemble the sliders, transversal sections of the sheets with a thickness of 5 µm were obtained with the aid of a table microtome brand LEICA SM 2000 R. For structural characterization, the samples were stained for 15 minutes in toluidine blue pH 4.0 (O'Brient & Mccully, 1981).

The slides were assembled with distilled water and the images captured using the photomicroscope (model LEICA DM 1000) with a camera system (model LEICA DFC 295) attached, and the images were photomicrographed with the aid of the Leica Application Suite software (Version 3.0). The photos of the leaves sections were stored in the Joint Photographic Experts Group (JPEG) format and the measurements were performed with the aid of the ImageJ software.

Statistical analysis

To differentiate each tissue from the leaves of *Aloysia citrodora*, they were evaluated as variables, among which: adaxial cuticle, abaxial cuticle, adaxial epidermis, abaxial epidermis, palisade parenchyma, lacunous parenchyma, leaf mesophyll (formed by a palisade and lacunous parenchyma), and leaf thickness, corresponding to the total thickness of the leaf (Figure 1). Besides, the mesophyll and the thickness of the main rib, and the length and width of the vascular system were also evaluated (Figure 1). The length was determined vertically over the vascular system while the width was performed transversely. The measurements were determined and expressed in micrometers (µm).

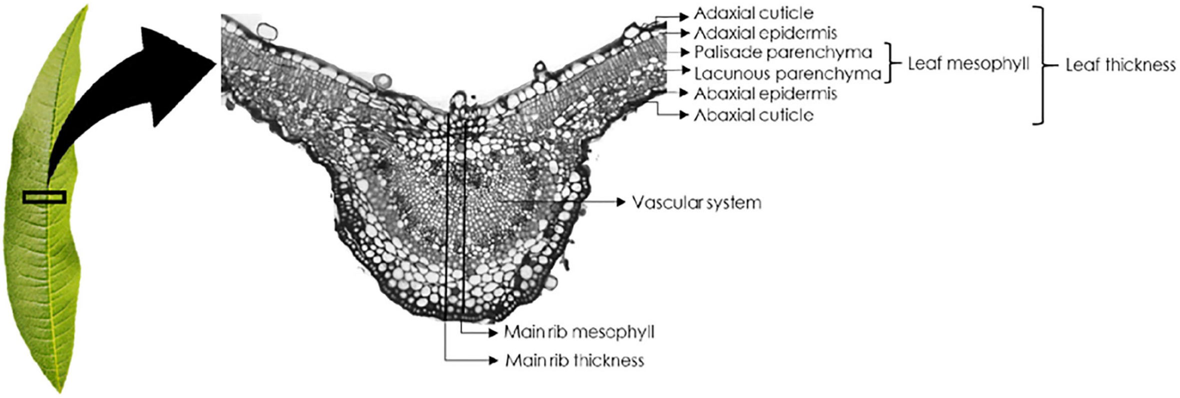

Figure 1. Graphical representation of the leaf position of *Aloysia citrodora* in which anatomical cuts were made and which tissues were evaluated.

The data were subjected to analysis of variance to assess the effect of seasonality and the different levels of water availability, and when significant, multiple comparisons of means was performed using the Scott and Knott test, at 5% probability of error, using the statistical program R - Package 'ExpDes' (Ferreira et al., 2014).

Results

The meteorological conditions during the period of the experiment inside the protected environment showed high thermal amplitude, with temperatures ranging from -2.1 to 47.9°C between the coldest (autumn season) and warmest (spring season) periods, respectively (Figure 2). In the autumn it was possible to observe maximum and minimum temperatures ranging from 41.5 and -2.1°C and the average temperature of the period was 19.9°C (Figure 2A). For the winter season, the average temperature was higher than in the autumn (23.2°C), but this period presented a high thermal amplitude with maximum and minimum temperatures of 46.6 and 2°C, respectively (Figure 2B). In the spring, temperatures were between 47.9 and 10.8°C with an average temperature of 28.7°C (Figure 2C). The average temperature for summer was 30°C, with maximum and minimum temperatures of 45.8 and 11.6°C, respectively (Figure 2D).

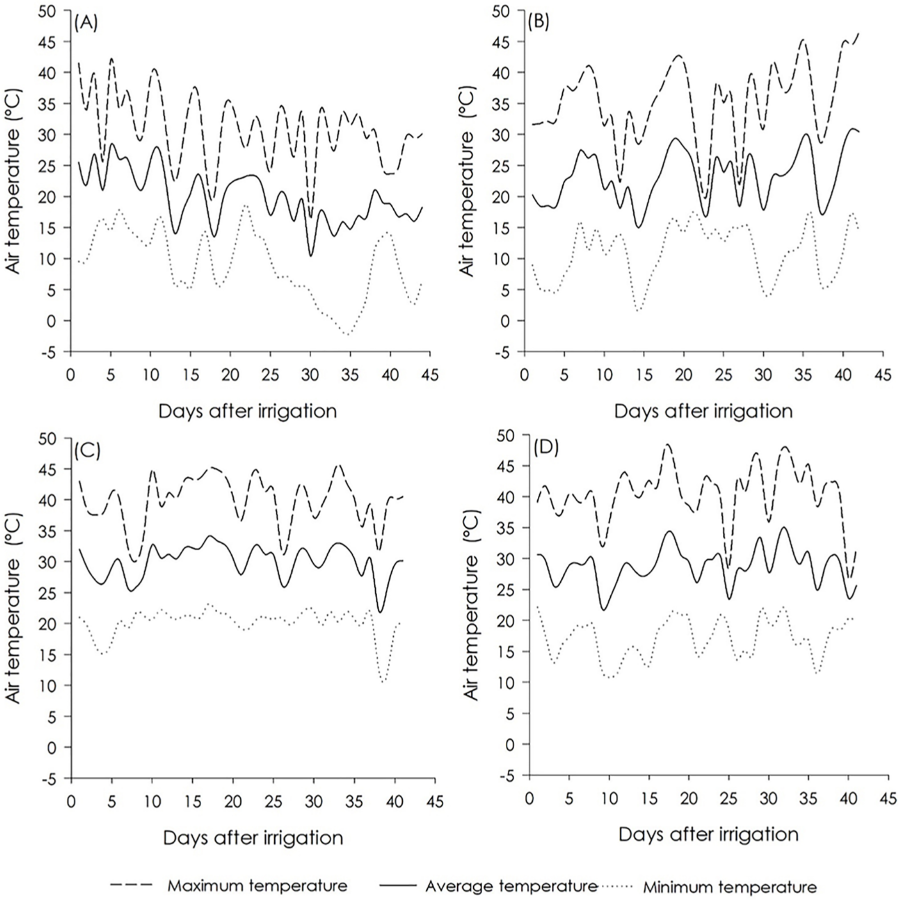

Figure 2. Maximum, minimum and average air temperatures recorded inside the protected environment in autumn (A), winter (B) and spring (C) of 2016 and summer (D) 2016/2017, when *Aloysia citrodora* was under different irrigation levels.

From the analysis of variance, it was observed that the measurements of the transversal section of the leaf referring to the adaxial cuticle, abaxial cuticle, adaxial epidermis, abaxial epidermis, palisade parenchyma, lacunous parenchyma, mesophyll, and leaf thickness were significant for the interaction between the factors seasonality x water availability, by the F test. For the measurements of the structures of the main rib of the leaf, there was a significant interaction between the factors seasonality x water availability, for the variables length of the vascular system and mesophilic and man rib thickness. The variable width of the vascular system of the main rib leaf showed a significant difference only for the seasonality factor.

From the leaf anatomy of *Aloysia citrodora* plants subjected to different water availability and seasonality, we observed several changes in the internal structures of the transversal sections and the main rib of the leaves (Figure 3).

For the adaxial cuticle of the transversal section of a leaf, there was a reduction in the cuticle thickness during the autumn season for the simulations of 75% and 100% of the field capacity, while for autumn and summer it reduced to 25% of the field capacity. When comparing water availability simulations within each season level, it was observed that there was a significant difference only for the summer, where 75% and 100% of the field capacity stimulated the formation of cuticle thickening with approximately 11.64 and 10.50 µm, respectively (Figures 3.30; 3.32 and 4A).

During the spring, and increased abaxial cuticle of plants in 25% field capacity, however, not differing from the 100% simulation (Figures 3.18 and 4B). However, under 100%, there was a reduction in the cuticle thickness (4.73 µm) of the leaves of the plants during the autumn season (Figures 3.8 and 4B). The winter season provided a reduction in the abaxial cuticle by 25% reaching 5.07 µm in thickness (Figures 3.10 and 4B).

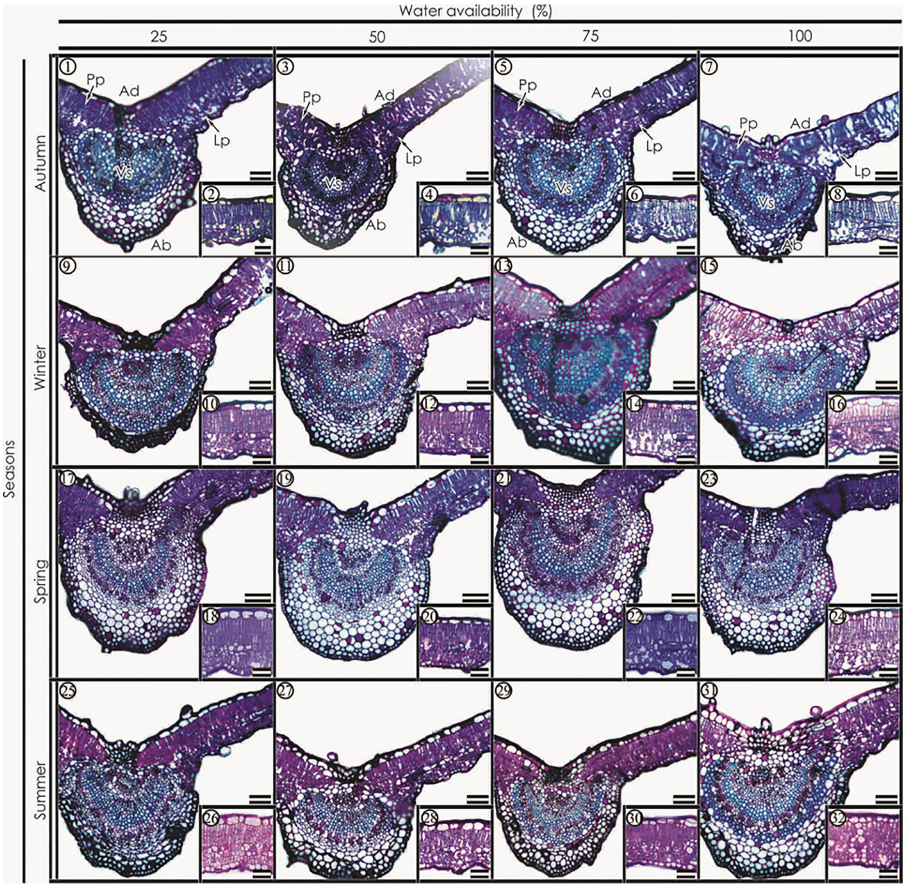

Figure 3. Transversal sections of *Aloysia citrodora* leaves, submitted to different simulations of water availability (25, 50, 75 and 100%) and seasons (autumn, winter, spring, and summer). Abbreviations: Ad: Adaxial epidermis; Pp: Palisade parenchyma; Lp: Lacunous parenchyma; Ab: Abaxial epidermis; Vs: Vascular system. Bars: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31= 100 µm; 2; 4; 6; 8; 10; 12; 14; 16; 18; 20; 22; 24; 26; 28; 30 and 32= 50 µm.

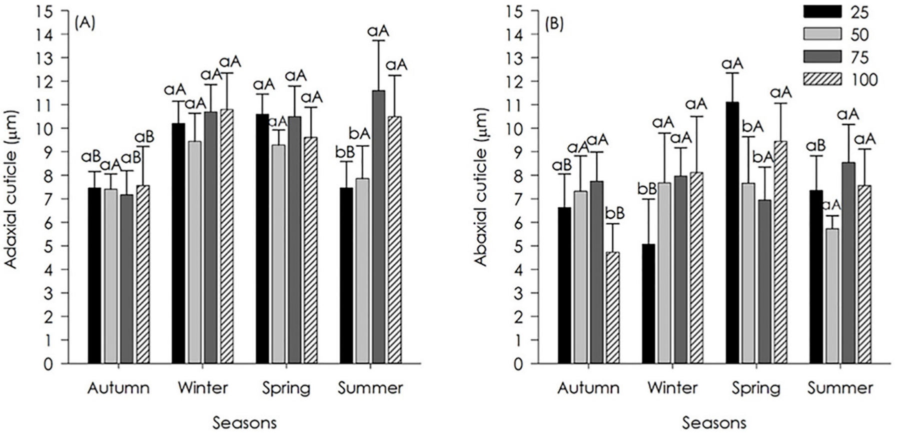

Figure 4. The thickness of the adaxial cuticle (A) and abaxial cuticle (B) of *Aloysia citrodora* leaves, submitted to different simulations of water availability (25, 50, 75, and 100% of the field capacity) and seasons (autumn, winter, spring, and summer). *Averages followed by the same capital letters between stations within each water availability simulation and lowercase letters between water availability within each station do not differ significantly, by the Scott and Knott test, at 5% probability of error. Vertical bars represent the standard deviation.

The adaxial epidermis showed significantly lower averages during the autumn when the plants remained underwater simulations of 50% and 100% of the field capacity, while it did not differ during the winter with 25% and in the spring with 75% of the field capacity (Figure 5A). When comparing the water simulations within each season, in the spring there was a greater thickness of the adaxial epidermis with 100% of the field capacity, with a thickness of 17.74 µm (Figures 3.24 and 5A).

During the winter season, there was a reduction in the thickness of the epidermis when the plants were submitted to 25% of the field capacity (Figures 3.10 and 5A), while there was no difference for the other seasons. For the abaxial epidermis, autumn also presented significantly lower averages when compared to other seasons in different water availability. However, in the spring with 100% of the field capacity, the plants showed an increase in the thickness of the abaxial epidermis (13.99 µm), while with 75% they presented a significant reduction in the value of this variable (Figures 3.24; 3.22 and 5B). In summer, the greatest thickening of the abaxial epidermis was observed with 25% and 100% of the field capacity (Figures 3.26; 3.32 and 5B).

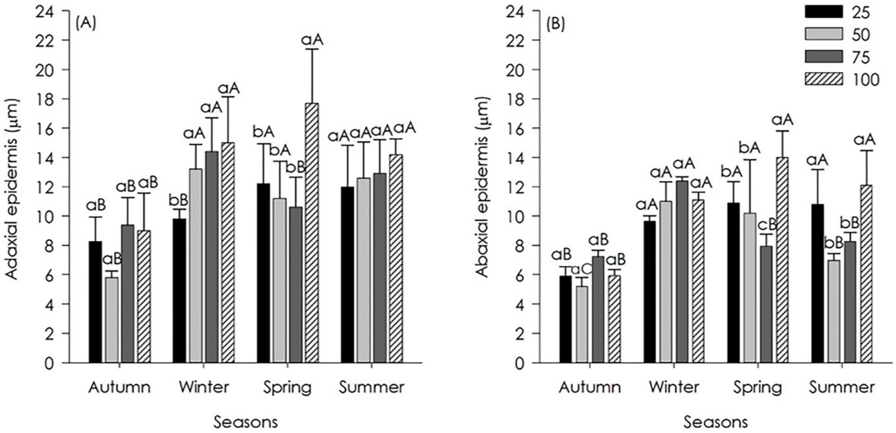

Figure 5. The thickness of the adaxial epidermis (A) and abaxial epidermis (B) of *Aloysia citrodora* leaves, submitted to different simulations of water availability (25, 50, 75, and 100% of the field capacity) and seasons (autumn, winter, spring, and summer). *Averages followed by the same capital letters between stations within each water availability simulation and lowercase letters between water availability within each station do not differ significantly, by the Scott and Knott test, at 5% probability of error. Vertical bars represent the standard deviation.

For the palisade parenchyma, it was observed that when the plants were under 25% of field capacity, there was a reduction in thickening when they were grown in the autumn and winter seasons; 75% when cultivated during the summer, and 100% of the field capacity during autumn and summer (Figure 6A). However, when comparing water availability within each season, a significant difference was observed only in the summer season, when lower water availability resulted in greater thickening of the palisade parenchyma (Figures 3.26 and 6A). The lacunous parenchyma showed a significant reduction in thickness when cultivated during autumn with water availability of 75% and during winter with 25% of field capacity (Figures 3.6; 3.10 and 6B).

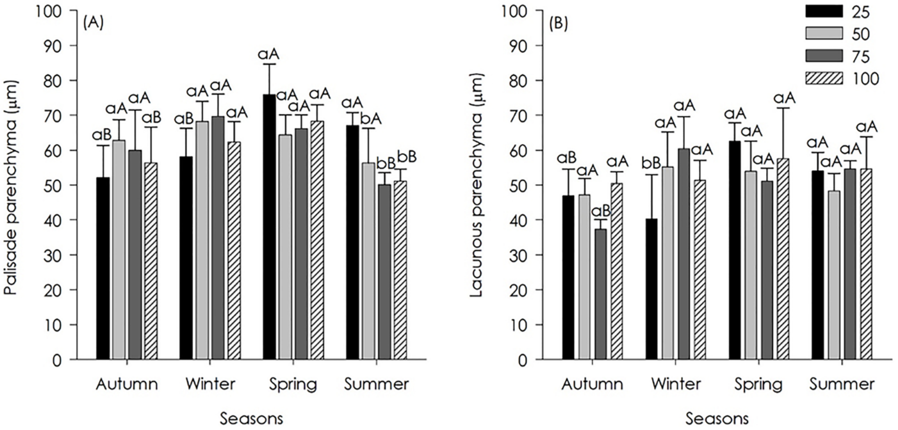

Figure 6. The thickness of palisade parenchyma (A) and lacunous parenchyma (B) of *Aloysia citrodora* leaves, submitted to different simulations of water availability (25, 50, 75, and 100% of the field capacity) and seasons (autumn, winter, spring, and summer). *Averages followed by the same capital letters between stations within each water availability simulation and lowercase letters between water availability within each station do not differ significantly, by the Scott and Knott test, at 5% probability of error. Vertical bars represent the standard deviation.

The leaf mesophyll of the transversal section of the leaf showed a similar response to the palisade and lacunous parenchyma, with a significant reduction during the autumn with water availability of 75% of the field capacity (Figures 3.4 and 7A). For winter, significantly lower results were observed in plants submitted to 25% of field capacity (Figures 3.10 and 7A). However, in summer there was a greater thickening of leaf mesophyll with less simulation of water availability (Figures 3.26 and 7A). Leaf thickness was severely affected with conditions of 25% of field capacity during the autumn and winter seasons (Figures 3.2; 3.10 and 7B). On the other hand, 25% of the field capacity, during the spring, provided greater leaf thickness (183.82 µm), not significantly differing from 100% of the field capacity (Figures 3.18; 3.24 and 7B).

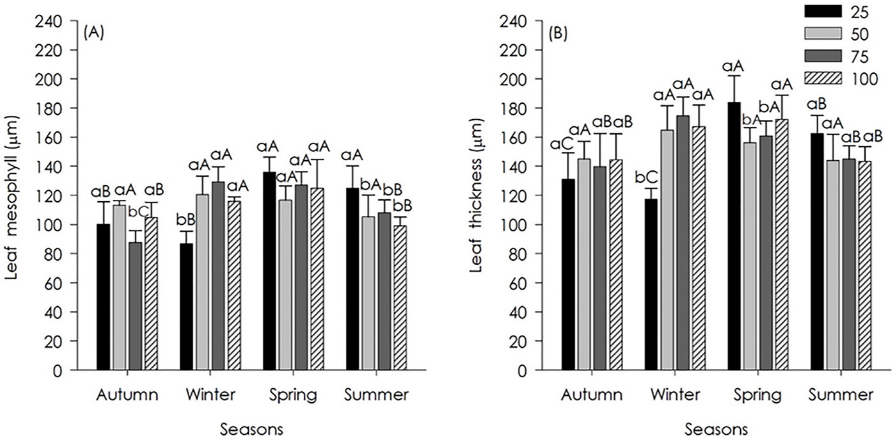

Figure 7. Mesophyll thickness (A) and leaf thickness (B) of *Aloysia citrodora* leaves, submitted to different simulations of water availability (25, 50, 75, and 100% of the field capacity) and seasons (autumn, winter, spring, and summer). *Averages followed by the same capital letters between stations within each water availability simulation and lowercase letters between water availability within each station do not differ significantly, by the Scott and Knott test, at 5% probability of error. Vertical bars represent the standard deviation.

The main rib mesophyll showed an increase in thickness when the plants were under the spring season when compared to the season within each water availability simulation (Figure 8A). In winter, there was a reduction in the mesophyll thickness of the plants maintained under the water regime of 25% of the field capacity while there was an increase in the mesophyll thickness of the rib under 75% during the spring season (Figures 3.9; 3.21 and 8A). In the same way, spring presented the greatest thickness of the main rib within each level of water availability. When assessing water availability within each season, there was a response like a leaf mesophyll, with a significant reduction in winter with 25% of field capacity and an increase of 75% during spring (Figures 3.9; 3.21 and 8B).

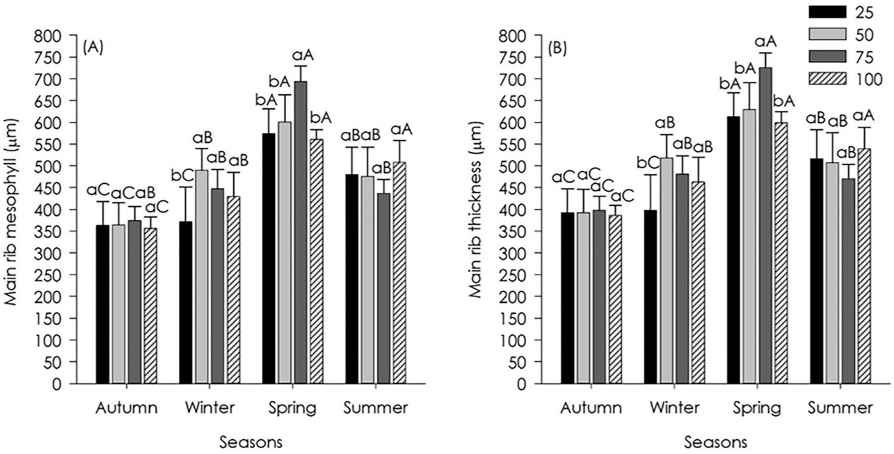

Figure 8. The thickness of the main rib mesophyll (A) and main rib thickness (B) of the transversal section of the median portion of *Aloysia citrodora* leaves, submitted to different simulations of water availability (25, 50, 75, and 100% of the field capacity) and seasons (autumn, winter, spring, and summer). *Averages followed by the same capital letters between stations within each water availability simulation and lowercase letters between water availability within each station do not differ significantly, by the Scott and Knott test, at 5% probability of error. Vertical bars represent the standard deviation.

The length of the vascular system of the main rib showed superiority during the spring season with 75% of the field capacity (Figures 3.21 and 9A). However, during autumn and summer, they showed a reduction in the length of the vascular system for most water availability (Figure 9A). For the variable width of the vascular system, there was a significant difference only for the seasonality factor, with significantly lower results for autumn (Figure 9B).

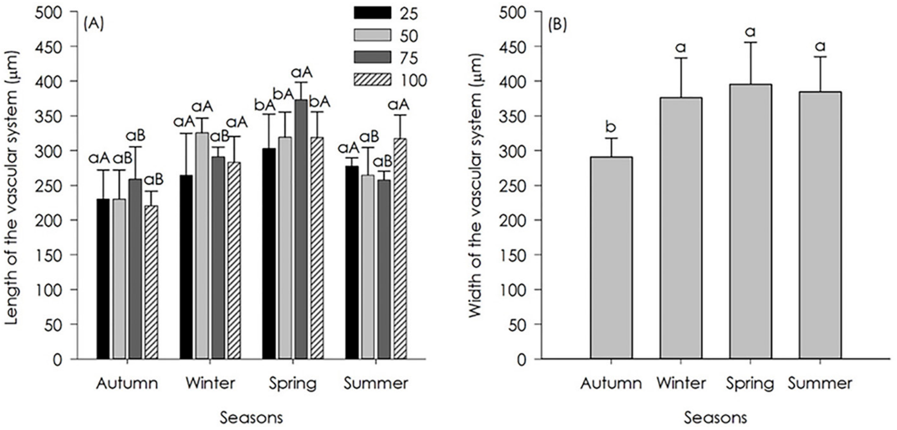

Figure 9. Measurements of the length (A) and width of the vascular system (B) of the main rib of *Aloysia citrodora* leaves, submitted to different simulations of water availability (25, 50, 75, and 100% of the field capacity) and seasons (autumn, winter, spring, and summer). *Averages followed by the same capital letters between stations within each water availability simulation and lowercase letters between water availability within each station do not differ significantly, by the Scott and Knott test, at 5% probability of error. Vertical bars represent the standard deviation.

Discussion

The morphological adaptations of plants to the environment act on the size and thickness of leaf structures of *Aloysia citrodora*, mainly observed when they are submitted to different environmental conditions. Under conditions of abiotic stress, the plant promotes anatomical changes, due to its plasticity, to tolerate stressors, and this study made it possible to characterize the internal morphology of the leaves of *Aloysia citrodora*, to identify anatomical changes according to the environmental conditions, confirming the plasticity observed in plants during water availability simulations that can occur during the seasons of a year of cultivation.

The *Aloysia citrodora* has a uniseriate epidermis, with only one cell layer on both sides of the leaf (abaxial and adaxial), with the function of coating and protecting the internal structures of the leaf. To withstand abiotic stresses, such as water stress, species, in general, have developed important strategies, mainly related to the changes that occur in the aerial part and result in changes in the size of the leaf and the thickness of the cuticle (Toscano et al., 2018).

These morphological changes in the cuticle were observed in the present work, mainly due to seasonality, which promotes changes in the environment and affects the plasticity of the plant. In the lowest water availability (25% of the field capacity), during the spring season and is the largest during the summer, there were the highest cuticle thicknesses, which can be justified as a defense mechanism against stressful conditions. Rosolem & Leite (2007) report that the leaf anatomy can vary depending on environmental factors such as solar radiation, temperature, water availability, among others, to avoid losses on their growth and development. Besides, the cuticle is a deposit of wax on the leaf surface, the thickness of which is a defense mechanism of the plant to prevent excessive water loss through the transpiration process (Baliza et al., 2012; Souza et al., 2010). Therefore, the greater cuticle thickness in the lower water availability during the spring, is related to the defense of the plant, either to avoid excessive water loss or as a form of thermal insulation and protection of the leaves, depending on the temperature variation (Figure 2) and solar radiation distinct throughout the seasons.

In addition to the cuticle perform the role of protecting the leaves, it is also responsible for the storage of secondary metabolites, such as essential oil, which is the main secondary metabolite produced by *Aloysia citrodora*. Thus, climatic variations, as well as water availability, can change the production of these metabolites (Oliveira et al., 2012), however, under stressful conditions plants tend to increase the production and storage of these substances, especially in the cuticle and glandular trichomes, as well as in other structures, which may influence the thickness of this anatomical variable.

The epidermis and cuticle are subject to structural changes depending on environmental conditions, as they are surfaces that make it difficult to lose water and increase leaf temperature. Thus, the increase in the thickness of the epidermis and cuticle of the adaxial face may also play a fundamental role in maintaining leaf temperature, due to the greater reflection of solar radiation, thus facilitating the photosynthetic process (Rossato & Kolb, 2010). When cutting the transversal section of *Ocimum gratissimum* leaves, the epidermis of the adaxial face is greater when compared to that of the abaxial face (Fernandes et al., 2014). These results corroborate those of the present study, since the adaxial epidermis of *Aloysia citrodora* is thicker when compared to the abaxial epidermis for all water availability and seasons, and this is justified due to the function of the adaxial face receiving greater solar radiation and the increase in epidermis thickness works as a protective barrier to not affect mesophyll structures.

In a study evaluating three levels of shading, Pinto et al. (2007) observed greater thickness of the adaxial epidermis, palisade parenchyma, lacunous parenchyma, and leaf thickness of *Aloysia gratissima* in the treatment with greater light intensity. However, in the present study greater thicknesses of the adaxial epidermis were observed in the seasons in which they present different conditions of luminosity and photoperiod (such as winter and spring, mainly), demonstrating that it is not only the meteorological elements that affect the morphological characteristics but also several other factors that work together, such as temperature, relative humidity, among others, in addition to the water availability evaluated in our study.

In general, the thickness of the abaxial epidermis, as well as of the lacunous parenchyma, observed in the present study, corroborate those observed by Pinto et al. (2007), when, working with *Aloysia gratissima* in cultivation under full sun, in which they observed thickness of 9.5 and 59.1 μm for epidermis and parenchyma, respectively. The thickness of the other tissues was different even though they were plants of the same genus since these thicknesses can be anatomical characteristics of each species.

The leaf mesophyll of *Aloysia citrodora* is characterized by the dorsiventral type, that is, it consists of palisade parenchyma in its upper portion (below the adaxial epidermis) and lacunous parenchyma in the lower portion, located between the palisade parenchyma and the abaxial epidermis of the leaf. The difference in the thickening of the palisade parenchyma is due to cell elongation, since the number of layers of this parenchyma was not affected, presenting itself in two layers. The increase in the thickness of the palisade parenchyma may occur due to the addition of new layers of this tissue, by the elongation of the cells or even by the combination of these two factors (Boeger et al., 2009).

The palisade parenchyma is related to photosynthesis, and the increase in the thickness of this tissue may be in response to favorable acclimatization, as it has a high amount of chloroplasts capable of increasing the CO_2 fixation and the photosynthetic rate in the leaves (Queiroz-Voltan et al., 2014). Therefore, in the spring, and summer the greatest thickness of the palisade parenchyma was observed in conditions of low water availability, as a form of tolerance in the face of these conditions. In seasons with milder temperatures (autumn and winter), the greatest thicknesses were obtained with water availability between 50 and 75% (even without a significant difference), demonstrating that both excess and water deficiency affects the thickness of these tissues. Thus, it can be justified that the effect of seasonality and water availability affects the thickness of the palisade parenchyma, and consequently, the photosynthetic rates, increasing or decreasing depending on environmental conditions.

When plants are exposed to adverse environmental conditions, such as seasonality and water availability, they can modify photosynthetic tissue and increase density, changes in intercellular spaces, and leaf mesophilic size to meet CO_2 demand and maintain water status in the plant (Lawson & Vialet-Chabrand, 2018). The lacunous parenchyma is related to the diffusion of CO_2, since the increase of this tissue increases the concentration gradient between the leaf air space and the atmosphere, increasing the competition for CO_2 (Fraser et al., 2008), which justifies the reduction of the thickness of this tissue observed during the winter in 25% water availability, which occurred since the plants reduce competition and diffusion of CO_2 in the leaves to tolerate a supposed water deficiency and acclimate to those conditions that are not favorable.

The leaf thickness is the result of the thickness of the epidermis and cuticles, together with the tissues of leaf mesophyll (palisade and lacunous parenchyma). Therefore, the greater the thickness of these tissues, the greater the thickness of the transversal section of the leaf. Toscano et al. (2018) in the evaluation of the physiological and anatomical responses of *Lantana camara* and *Ligustrum lucidum* leaves under different water availability, observed that water deficiency induced a reduction in leaf thickness, and these results are mainly related to the reduction of the palisade parenchyma. In the present study, contradictory results were observed in this study, because during the spring and summer seasons (preferential growing seasons), the lower water availability showed greater leaf thickness, resulting from the greater thickness of the palisade parenchyma (Figures 3.18 and 3.26, respectively). For autumn and winter, the reduction in water availability promoted a reduction in leaf thickness (Figure 3.10 and 3.2, respectively), however, for these seasons, the lacunous parenchyma was the tissue that most influenced leaf thickness.

The greatest leaf thickening is related to the thickness of the palisade and lacunous parenchyma (Fernandes et al., 2014). This statement corroborates the results found since the palisade and lacunous parenchyma were the main tissues responsible for defining the thickness of the transversal section of the leaf. However, the thickness of the palisade and lacunous parenchyma were quite variable during the growing seasons, and the palisade parenchyma positively influenced the leaf thickness, regardless of the environmental condition in which the plants were submitted.

In *Arbutus unedo* and *Phillyrea angustifolia*, the highest leaf thickness was observed during autumn and winter crops, when compared to spring and summer seasons, in evaluations related to seasonality and responses to ultraviolet radiation and precipitation regimes (Verdaguer et al., 2018). This partially corroborates those observed in the present, as only for the winter with water availability of 75% and 100% of the field capacity that was superior to the summer. However, leaf thickness in these water conditions did not differ between the winter and spring seasons, demonstrating that leaf thickness is characteristic for each species and variable depending on the environmental conditions in which the plants are exposed.

In the main rib, a high amount of vascular tissue and parenchymal tissue can be observed. The vascular system is in the center of the vein and is collateral, with the xylem facing the adaxial epidermis and phloem to the abaxial, responsible for vascularization throughout the leaf blade. The increase in the thickness of the main rib of the leaf may be due to the enlargement of the filling tissues and the conductive or vascular tissues (Queiroz-Voltan et al., 2014), constituted by xylem and phloem. The mesophyll and main rib thickness of the median transversal section of leaves of *Aloysia citrodora* showed variations between seasons and under different water availability, indicating different anatomical characteristics for each condition. The greater thickness of the mesophyll and the rib observed during spring in practically all water availability can be related to the better environmental conditions of this period, since it is a plant that presents greater vegetative growth in mid-october (Brant et al., 2008). Also, the greatest thicknesses were not obtained under conditions of high water availability (100%), but in the condition of 75% of the field capacity, demonstrating that water availability influences the anatomy of the transversal section of the median portion of the leaf.

On the other hand, the autumn season negatively affected the thickness of the mesophyll and the thickness of the transversal section of the median portion of the leaf, which can be justified due to the reduced length and width of the vascular system, when compared to the other seasons. However, the variable width of the vascular system was not influenced by water availability, only by the effect of seasonality. Thus, even without a significant difference between the other seasons, it is worth noting that the thickness of the main leaf vein is related to the thickness of the vascular system. Queiroz-Voltan et al. (2014) also point out that in *Coffea arabica*, the thickness of the vascular system influences the phloem sap flow, and the greater the thickening of the vascular system, the greater the sap flow and the better the acclimatization during water restriction conditions. Therefore, the greatest thickening of the vascular system found during the winter, spring, and summer seasons demonstrates a characteristic of acclimatization of the plants or mirrors the environmental conditions more favorable to the growth and development of *Aloysia citrodora*.

In summary, the autumn season reduced the width of the vascular system and the thickness of the adaxial cuticle under greater water availability simulation and, when compared to other seasons, reduced most leaf tissues. When the plants remain during the winter season under low water availability, they reduce the thickness of the adaxial cuticle, adaxial epidermis, lacunous parenchyma, mesophyll and leaf thickness, and mesophyll and main rib thickness. For spring cultivation under the greatest water availability resulted in an increase in the adaxial and abaxial epidermis, mesophyll, main rib thickness, and length of the vascular system. However, under low water availability, they provided a greater increase in the thickness of the adaxial cuticle, palisade parenchyma, and leaf thickness. During the summer and under high water availability, the leaf and adaxial cuticle thickness increases and reduces the palisade parenchyma, while under low water availability there is an increase in the thickness of the leaf mesophyll and reduction of the adaxial cuticle.

Conclusions

This is the first report of the impact of water availability and the season under the different leaf tissues of *Aloysia citrodora*. Therefore, it is suggested that the spring and summer seasons with greater water availability are likely to increase phytomass and essential oil yield. On the other hand, the winter with less water availability may lead to less accumulation of phytomass, but possibly an increase in the essential oil content, due to the stressful conditions, which suggests the need for future work to confirm these hypotheses.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Moraes, G., Leonardo, J., Sparovek, G. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711-728.

Álvarez, S., Navarro, A., Nicolás, E., Sánchez-Blanco, M.J. 2011. Transpiration, photosynthetic responses, tissue water relations and dry mass partitioning in *Callistemon* plants during drought conditions. *Scientia Horticulturae* 129: 306-312.

Bahramsoltani, R., Rostamiasrabadi, P., Shahpiri, Z., Marques, A.M., Rahimi, R., Farzaei, M.H. 2018. *Aloysia citrodora* Palau (Lemon verbena): A review of phytochemistry and pharmacology. *Journal of Ethnopharmacology* 222: 34-51.

Baliza, D.P., Cunha, R.L., Castro, E.M., Barbosa, J.P.R.A.D., Pires, M.F., Gomes, R.A. 2012. Trocas gasosas e características estruturais adaptativas de cafeeiros cultivados em diferentes níveis de radiação. *Coffee Science* 7: 250-258.

Batista, L.A., Guimarães, R.J., Pereira, F.J., Carvalho, G.R., Castro, E.M. 2010. Anatomia foliar e potencial hídrico na tolerância de cultivares de café ao estresse hídrico. *Revista Ciência Agronômica* 41: 475-481.

Bisbis, M.B., Gruda, N., Blanke, M. 2018. Impactos potenciais das mudanças climáticas na produção vegetal e na qualidade do produto - Uma revisão. *Journal of Cleaner Production* 170: 1602-1620.

Boeger, M.R.T., Espíndola Júnior, A., Maccari Júnior, A., Reissmann, C.B., Alves, A.C.A., Rickli, F.L. 2009. Variação estrutural foliar de espécies medicinais em consórcio com erva-mate, sob diferentes intensidades luminosas. *Floresta* 39: 215-225.

Brant, R.S., Pinto, J.E.B.P., Bertolucci, S.K.V., Albuquerque, C.J.B. 2008. Teor do óleo essencial de cidrão [*Aloysia triphylla* (LHér.) Britton] em função da variação sazonal. *Revista Brasileira de Plantas Medicinais* 10: 83-88.

Du, Q., Liu, T., Jiao, X., Song, X., Zhang, J., Li, J. 2019. Leaf anatomical adaptations have central roles in photosynthetic acclimation to humidity. *Journal of experimental botany* 70: 4949-4962.

Fang, Y., Xiong, L. 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences* 72: 673-689.

Fernandes, V.F., Bezerra, L.D.A., Mielke, M.S., Silva, D.D.C., Costa, L.C.D.B. 2014. Leaf anatomy and ultrastructure of *Ocimum gratissimum* under different light radiation levels. *Ciência Rural* 44: 1037-1042.

Ferreira, E., Cavalcanti, P. Nogueira, D. 2014. ExpDes: An R Package for ANOVA and Experimental Designs. *Applied Mathematics* 5: 2952-2958.

Fraser, L.H., Greenall, A., Carlyle, C., Turkington, R., Friedman, C.R. 2008. Adaptive phenotypic plasticity of *Pseudoroegneria spicata*: response of stomatal density, leaf area and biomass to changes in water supply and increased temperature. *Annals of Botany* 103: 769-775.

Grisi, F.A., Alves, J.D., Castro, E.D., Oliveira, C.D., Biagiotti, G., Melo, L.D. 2008. Avaliações anatômicas foliares em mudas de café 'Catuaí'e 'Siriema' submetidas ao estresse hídrico. *Ciência e Agrotecnologia* 32: 1730-1736.

Gratani, L. 2014. Plant Phenotypic Plasticity in Response to Environmental Factors. *Advances in Botany* 2014.

Isah, T. 2019. Stress and defense responses in plant secondary metabolites production. *Biological research* 52: 39.

Lawson, T., Vialet-Chabrand, S. 2019. Speedy stomata, photosynthesis and plant water use efficiency. *New Phytologist* 221: 93-98.

Lee, H., Feakins, S.J., Sternberg, L.S.L. 2016. Carbon and hydrogen isotopic effects of stomatal density in *Arabidopsis thaliana*. *Geochimica et Cosmochimica Acta* 179: 275-286.

Litvin, A.G., Van Iersel, M.W., Malladi, A. 2016. Drought stress reduces stem elongation and alters gibberellin-related gene expression during vegetative growth of tomato. *Journal of the American Society for Horticultural Science* 141: 591-597.

Makbul, S., Güler, N.S., Durmuş, N., Güven, S. 2011. Changes in anatomical and physiological parameters of soybean under drought stress. *Turkish Journal of Botany* 35: 369-377.

Martins, J.R., Alvarenga, A.A., Castro, E.M., Oliveira da Silva, A.P., Alves, E. 2010. Teores de pigmentos fotossintéticos e estrutura de cloroplastos de Alfavaca-cravo cultivadas sob malhas coloridas. *Ciência Rural* 40: 64-69.

Matesanz, S., Gianoli, E., Valladares, F. 2010. Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences* 1206: 35-55.

Melo Júnior, J.C.F., Boeger, M.R.T. 2016. Leaf traits and plastic potential of plant species in a light-edaphic gradient from restinga in southern brazil. *Acta Biológica Colombiana* 21: 51-62.

O'Brien, T.O., McCully, M.E. 1981. *The study of plant structure: principles and selected methods*. Thermarcarphi Pty. Ltd. Melbourne, Austrália, 345 p.

Oliveira, A.R.M.F., Jezler, C.N., Oliveira, R.A., Costa, L.C.B. 2012. Influência da idade da planta na produção de óleo essencial de alevante. *Revista Ceres* 59: 241-245.

Oz, M.T., Eyidogan, F., Yucel, M., Öktem, H.A. 2015. Functional role of nitric oxide under abiotic stress conditions. *Nitric Oxide Action in Abiotic Stress Responses in Plants* 1: 21-41.

Pinto, J.E.B.P., Cardoso, J.C.W., Castro, E.M., Bertolucci, S.K., Melo, L.A., Dousseau, S. 2007. Aspectos morfofisiológicos e conteúdo de óleo essencial de plantas de alfazema-do-Brasil em função de níveis de sombreamento. *Horticultura Brasileira* 25: 210-214.

Queiroz-Voltan, R.B., Nardin, C.F., Fazuoli, L.C., Braghini, M.T. 2014. Caracterização da anatomia foliar de cafeeiros arábica em diferentes períodos sazonais. *Biotemas* 27: 1-10.

Rosolem, C.A., Leite, V.M. 2007. Coffee leaf and stem anatomy under boron deficiency. *Revista Brasileira de Ciência do Solo* 31: 477-483.

Rossato, D.R., Kolb, R.M. 2010. *Gochnatia polymorpha* (Less.) Cabrera (Asteraceae) changes in leaf structure due to differences in light and edaphic conditions. *Acta Botanica Brasilica* 24: 605- 612.

Schöttler, M.A., Tóth, S.Z. 2014. Photosynthetic complex stoichiometry dynamics in higher plants: environmental acclimation and photosynthetic flux control. *Frontiers in Plant Science* 5: 1-15.

Souza, T.C.D., Magalhães, P.C., Pereira, F.J., Castro, E.M.D., Silva Junior, J.M.D., Parentoni, S.N. 2010. Leaf plasticity in successive selection cycles of 'Saracura' maize in response to periodic soil flooding. *Pesquisa Agropecuária Brasileira* 45: 16-24.

Tenhaken, R. 2015. Cell wall remodeling under abiotic stress. *Frontiers in Plant Science* 5: 1-9.

Toscano, L., Ferrante, A., Tribulato, A., Romano, D. 2018. Leaf physiological and anatomical responses of *Lantana* and *Ligustrum* species under diferente water availability. *Plant Physiology and Biochemistry* 127: 380-392.

Vasellati, V., Oesterheld, M., Medan, D., Loreti, J. 2001. Effects of flooding and drought on the anatomy of *Paspalum dilatatum*. *Annals of Botany* 88: 355-360.

Verdaguer, D., Díaz-Guerra, L., Font, J., González, J. A., Llorens, L. 2018. Contrasting seasonal morphological and physio-biochemical responses to UV radiation and reduced rainfall of two mature naturally growing Mediterranean shrubs in the context of climate change. *Environmental and Experimental Botany* 147: 189-201.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.