

Artículo Original / Original Article

Physiological and metabolic alterations in basil (*Ocimum basilicum* L.) varieties under distinct soil water levels

[Alteraciones fisiológicas y metabólicas en variedades de albahaca (*Ocimum basilicum* L.) bajo distintos niveles de agua del suelo]

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Abstract: Basil (*Ocimum basilicum* L.) is a medicinal species used in several areas, such as food, medicines and cosmetics, and the understanding of its physiological behavior under environmental conditions is of paramount importance for the improvement of cultivation methods. The objective of this study was to evaluate the influence of different water availability under physiological, biochemical and metabolic characteristics, in three distinct genotypes: 'Alfavaca basiliçãõ', 'Gennaro de menta' and 'Grecco à palla', during two different phenological stages (vegetative and reproductive). It was found that the water deficit promotes physiological changes to tolerate water stress, and the studied genotypes have different routes to achieve this physiological tolerance, which culminates in a distinct accumulation of metabolites in plants, and can be considered interesting if the final product is the production of essential oils.

Keywords: Irrigation; Plant metabolism; Essential oils; Medicinal plants; *Ocimum basilicum*.

Resumen: La albahaca (*Ocimum basilicum* L.) es una planta medicinal utilizada en varias áreas: alimenticia, medicinal e industria cosmética; es de suma importancia el entendimiento de su comportamiento fisiológico bajo diferentes condiciones ambientales con el fin de mejorar los procesos del cultivo. El objetivo de este estudio fue evaluar la influencia de diferentes disponibilidades hídricas en las características fisiológicas, bioquímicas y metabólicas en tres genotipos de albahaca: "Alfavaca basiliçãõ", "Gennaro de menta" y "Grecco à palla" durante dos etapas fenológicas (vegetativa y reproductiva). Fue encontrado que el déficit hídrico promueve cambios fisiológicos con el fin de tolerar el estrés hídrico. Los genotipos estudiados presentaron diferentes rutas para alcanzar esta tolerancia fisiológica, la cual culmina con distintas acumulaciones de metabolitos en las plantas, y puede ser considerado interesante si el producto final es la producción de aceites esenciales.

Palabras clave: Riego; Metabolismo vegetal; Aceites esenciales; Plantas medicinales; *Ocimum basilicum*

INTRODUCTION

Basil (*Ocimum basilicum* L.) stands out as an important medicinal and aromatic plant, thanks to the main components (linalool and eugenol) present in its essential oil (Dou *et al.*, 2018; Dou *et al.*, 2019), with multiple uses from formulated for agricultural applications to cosmetic use (Dorni *et al.*, 2017). However, all its productive potential of biomass and chemical compounds is strongly conditioned by the genotypes used and by the environmental conditions of cultivation (Paton & Putievsky, 1996; Patel *et al.*, 2016).

Among the environmental conditions that most impact basil, the availability of water is a major factor for this species, studies show that water stress negatively affects physiological processes and, consequently, plant growth (Afkari, 2018). The water deficit restricts the stomatal opening, by reducing the water potential in the leaf and its stomatal conductance, resulting in the blockage of CO₂ flow, reduction in the accumulation of photoassimilates and reducing productivity (Basu *et al.*, 2016, Bianchi *et al.*, 2016).

For *Ocimum* cultivation, water stress has deleterious effects on physiological, morphological and biochemical characteristics (Pirbalouti *et al.*, 2017), however, since it is an aromatic plant, many oils of commercial interest are produced in secondary metabolism routes, and in basil, the water deficit causes increases in these constituents to a certain point of deficient water control (Omidbaigi *et al.* 2003).

This research had as main objective to evaluate the effect of different levels of water availability on the physiological, biochemical and metabolic performance, in three varieties of *O. basilicum* (Alfavaca Basilicão, Gennaro de Menta and Grecco à palla) during the vegetative and reproductive phase of the plants.

MATERIAL AND METHODS

Experiment location and conditions

The research was conducted in a greenhouse and in the Plant Production Physiology and Metabolism laboratory (Laboratório de Fisiologia e Metabolismo da Produção Vegetal — LAFIMEPRO), both at the Federal University of Viçosa - Rio Paranaíba Campus, from September 2018 to February 2019, covering a period of 125 days. The plants of *Ocimum basilicum* L. were obtained through propagation via

seeds (Isla Sementes®, Brazil), of three varieties of basil (Alfavaca basilicão, Gennaro de menta and Grecco à palla). The seeds were grown in polyethylene trays of 128 cells, filled with commercial substrate (Plantmax®), and after 28 days of planting (DAP), the plants were transplanted into plastic pots (5 dm³) filled with clayey oxisol, with the following chemical characteristics: Organic Matter: 26 g dm⁻³; P: 8 mg dm⁻³; K: 2,2 mmol dm⁻³; Ca: 33 mmol dm⁻³; Mg: 10 mmol d⁻³; pH: 5,3. Fertilization was carried out during transplanting with 30 g m⁻² of fertilizer in formulation 08-28-16 (N-P-K). Cultivation activities were carried out in accordance with the recommended practices for culture.

The irrigation of the pots was performed according to the maximum water retention capacity of the soil, proposed by Fernandes and Syke (1968). 80% of field capacity was considered an adequate condition for water volume and 60% of field capacity was a condition of moderate scarcity. From planting to the imposition of stresses, the pots were kept under irrigation of 80% of the field capacity, with daily monitoring to replace the water evapotranspiration.

The experiment was divided into two stages, with an evaluation of the behavior of the plants during the vegetative phase (75 to 78 DAP) and in the flowering stage (120 to 123 DAP). Both stages were evaluated under two water availability regimes, being 60% (moderate stress) and 80% (no stress) of the water capacity in the soil.

First Stage

Relative water content (RWC)

It was determined during the vegetative and flowering stages. 10 leaf discs were removed and the fresh weight (FW) was recorded immediately. The leaf discs were kept in 20mL of water for 24 hours and, subsequently, the wet weight (WW) was obtained. Then, the plant material was dried for 72 hours at 70°C in an oven and the dry weight (DW) was recorded. The RWC was calculated using the formula:

$$RWC(\%) = \left[\frac{FW - DW}{WW - DW} \right] \times 100$$

Total soluble protein (TSP)

It was determined during the vegetative stage using the method described by Bradford (1976). The reaction was prepared with 20 µL of the protein

extract, added to 3.0 mL of Bradford's reagent, (LGC Biotecnologia, Cotia, SP, Brazil). The reading was performed on a spectrophotometer at 595 nm, and the TSP concentration was calculated from the standard curve prepared with bovine serum albumin (BSA). The results obtained were expressed in mg of protein per g⁻¹ of fresh matter.

Activity of antioxidant enzymes and proline

The quantification of the activity of ascorbate peroxidase (APX) and catalase (CAT) were evaluated in a vegetative stage. APX activity was determined by the method described by Nakano and Asada (1981), and the results were expressed in $\mu\text{mol APX min}^{-1} \text{mg}^{-1} \text{TSP}$. CAT activity was determined by the method described by Havir and McHale (1987), and the results were expressed in $\mu\text{mol CAT min}^{-1} \text{mg}^{-1} \text{TSP}$. The proline content was quantified according to Bates *et al.* (1973) during the vegetative and flowering stages.

Photosynthetic pigments content

The quantification of chlorophyll *a* (*Chl. A*), chlorophyll *b* (*Chl. B*), total chlorophyll (*Chl. Total*) and carotenoids (*Car.*) were evaluated in vegetative stage and flowering through solvent extraction (80% acetone), modified according to Macedo *et al.* (2013). The plant material was placed in plastic tubes (50mL) containing an 80% (v/v) acetone solution and were kept in the dark for 72 hours. Subsequently, the extracts were read on a spectrophotometer (SP-2000 UV Spectrum) at absorbances (Witham *et al.*, 1971) of 663, 645 and 470 nm, to determine chlorophylls *a*, *b* and carotenoids, respectively. The values obtained were used to calculate the results, and these were expressed in milligrams per gram of pigment in fresh leaf tissue (mg g⁻¹).

Essential oils

It was performed using the hydrodistillation method, using a CLEVANGER apparatus, using 20 g of the dry leaves of plants in a vegetative stage mixed in 400 mL of distilled water, the extraction time was 90 minutes, after the first drop. The chemical analysis of essential oils was by gas chromatography coupled to mass spectrometry (GC-MS), in a QP2010 model equipment (Shimadzu). The compounds were identified by comparing the mass spectra with those in the NIST library, visual interpretation of the mass spectra and comparing the retention indices. The

relative percentage of each compound was calculated using the ratio between the area of each peak and the total area of all constituents in the sample.

Second Stage

It consisted of the reanalysis of the RWC, proline and photosynthetic pigments, as previously presented, in addition to analysis of gas exchange and extravasation of electrolytes in leaf tissues for the flowering stage.

Gas exchange

The evaluations included the measurement of the CO₂ assimilation rate (*A*) ($\mu\text{mol m}^{-2} \text{s}^{-1}$), leaf transpiration (*E*) ($\text{mmol of H}_2\text{O m}^{-2} \text{s}^{-1}$) and stomatal conductance (*gs*) ($\text{mmol m}^{-2} \text{s}^{-1}$). In possession of these data, the water use efficiency (*A/E*) [$(\mu\text{mol m}^{-2} \text{s}^{-1}) / (\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1})$] was calculated. The measurements took place during the three days of water restriction, from 9:00 am to 10:00 am, with the gas exchange analyzer, model LI-6400 XT (Li-Cor Biosciences, USA), coupled to a fluorometer, with photon flow density of 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$. No gas exchange assessments were carried out for the Gennaro de menta variety, due to the reduced size of the leaf area.

Electrolyte leakage (EL)

The method proposed by Blum and Ebercon (1981), adapted by Silveira *et al.* (2001). The electrical conductivity (L1) of a distilled water solution (20mL) containing 10 leaf discs immersed for 24 hours was determined. The samples remained in a water bath at 100°C for one hour and, subsequently, the electrical conductivity of the solution was measured again (L2). The quantification (%) of the extravasation of electrolytes was obtained using the formula:

$$EL(\%) = \left(\frac{L1}{L2} \right) \times 100$$

Statistical analysis

The experimental design used was completely randomized, in a 3x2 factorial scheme, being: three varieties of basil - Gennaro de Menta, Grecco à palla and Alfavaca basilição - and two water levels - 60% (moderate stress) and 80% (no stress) of maximum water retention capacity in the soil -, with four repetitions. Subsequently, the data obtained were subjected to analysis of variance (ANOVA) and comparisons between averages by the Student-

Newman-Keuls test (SNK) at 5% probability, using the statistical program *Speed Stat* (Carvalho & Mendes, 2017). While for the analysis of essential oils, the means were evaluated by principal component analysis (PCA), with the *PAST* software (Hammer et al., 2001).

RESULTS

Relative water content

The results obtained for the relative water content (RWC) in the vegetative phase (Figure No. 1), demonstrated that at the level of 80% and 60% of water in the soil there was no significant difference

between the varieties. However, the Gennaro de menta variety, when subjected to 60% water level, significantly decreases the RWC. For the RWC in the flowering phase (Figure No. 1), there was no significant difference between the varieties at the level of 80% of water, however at the level of 60% of water in the soil, the varieties showed different behavior, the Gennaro de menta being the material that best managed to stabilize the water content in the leaves, while the Alfavaca basilicão and Grecco à palla showed significant reductions to the detriment of the water deficit.

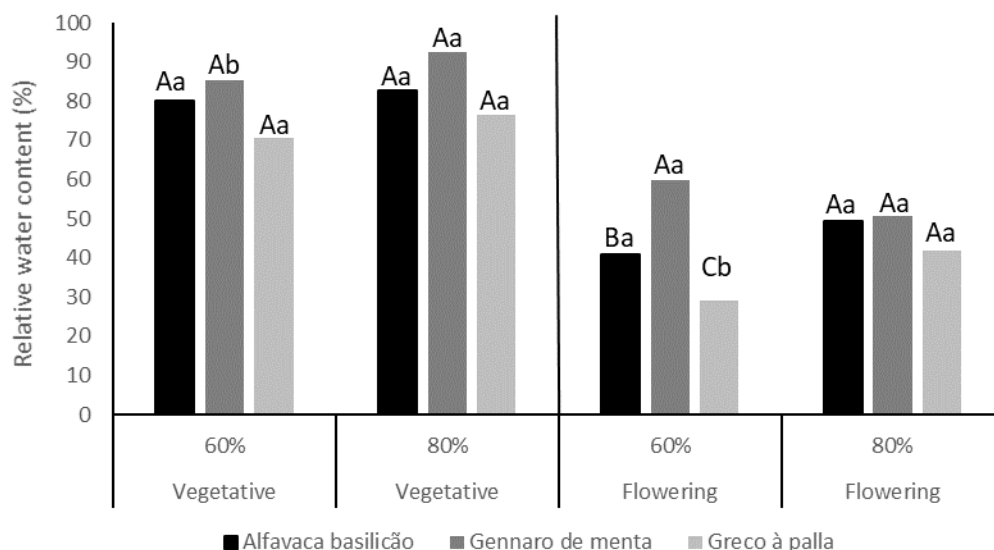


Figure No. 1

Relative water content in leaves at 78 (A) and 123 (B) DAP. Averages followed by the same letter do not differ statistically by the SNK test (5%). Uppercase letters compare the averages within the water levels, and lowercase letters compare the averages within the varieties, at the two evaluated stages

Photosynthetic pigments

During the vegetative stage, there was no significant difference between the varieties in the two water levels for the variable chlorophyll *a* content (Figure No. 2A), however the varieties Gennaro de menta and Grecco à palla showed lower contents at the level of 60% water. As for the content of chlorophyll *b* (Figure No. 2B), in the vegetative phase, the water deficit reduced the content in the varieties Alfavaca basilicão and Grecco à palla when compared to Gennaro de menta. In the flowering phase, the opposite occurred, with the Gennaro de menta variety

having a lower chlorophyll content than the other varieties and with itself at 80% water level (Figure No. 2). And when analyzing the total chlorophyll content (Figure No. 2C), in the vegetative phase, it was found that the moderate stress decreased significantly for Grecco à palla, while for plants that were not subjected to stress, there were no changes in the content. In the flowering stage, the Gennaro de menta variety showed a reduction in its total chlorophyll at the level of 60% of water in the soil, when compared with the other varieties and with the level of 80% of water in the soil. As for the

carotenoid content (Figure No. 2D), it was observed significant differences in the vegetative phase for Grecco à palla, with significant reductions when compared between water levels, as in cultivars, while in the flowering stage the Gennaro de menta variety showed lower content than the other varieties and

when compared to the level of 80% of water in the soil. It was found that the levels of photosynthetic pigments were influenced by the water deficit both in the vegetative phase and in the flowering phase, indicating the flexibility of each variety under the two water levels.

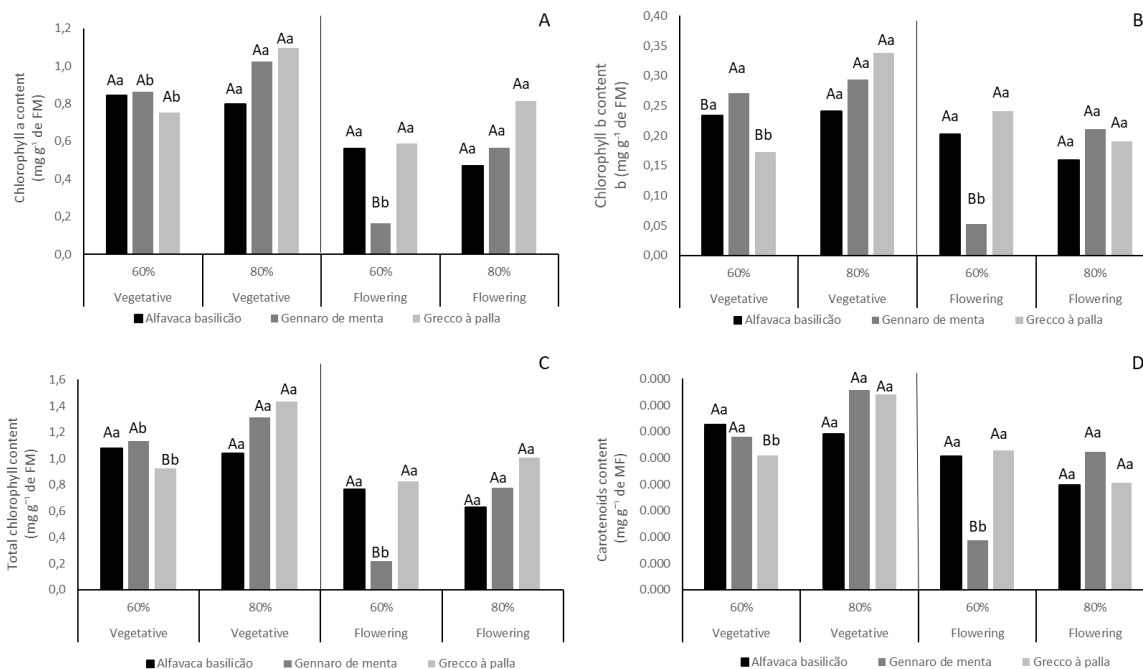


Figure No. 2

Content of photosynthetic pigments, chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D), in basil leaves at 78 DAP and 123 DAP. Averages followed by the same letter do not differ statistically by the SNK test (5%). Uppercase letters compare the averages within the water levels, and lowercase letters compare the averages within the varieties, at the two evaluated stages

Total soluble protein, antioxidant enzymes and proline

For the content of total soluble proteins (Figure No. 3A), the results did not show significant interaction, between the water levels and the varieties under study, however, the Gennaro de menta variety showed a significant reduction in the content of total soluble proteins when submitted to the level 80% water in the soil. In the analysis of the antioxidant enzymes APX (Figure No. 3B) and CAT (Figure No. 3C) it is noted that the enzymes have their activity increased for the varieties Alfavaca basilição and Gennaro de menta, while the material Grecco à palla reversed the behavior and showed to have more

activity of these enzymes at the level of 80% water availability in the soil.

For proline content, another molecular indicator of stress in plant tissues, significant increases were observed at the level of 60% water availability in the soil, with increments of 860%, 935% and 2430%, respectively, for Alfavaca basilição, Gennaro de menta and Grecco à palla (Figure No. 3D). Among the varieties, Grecco à palla presented the highest production of proline, followed by Gennaro de menta and Alfavaca basilição, indicating that the material has a highly specialized mechanism for the maintenance of cell turgor, when compared to the other varieties.

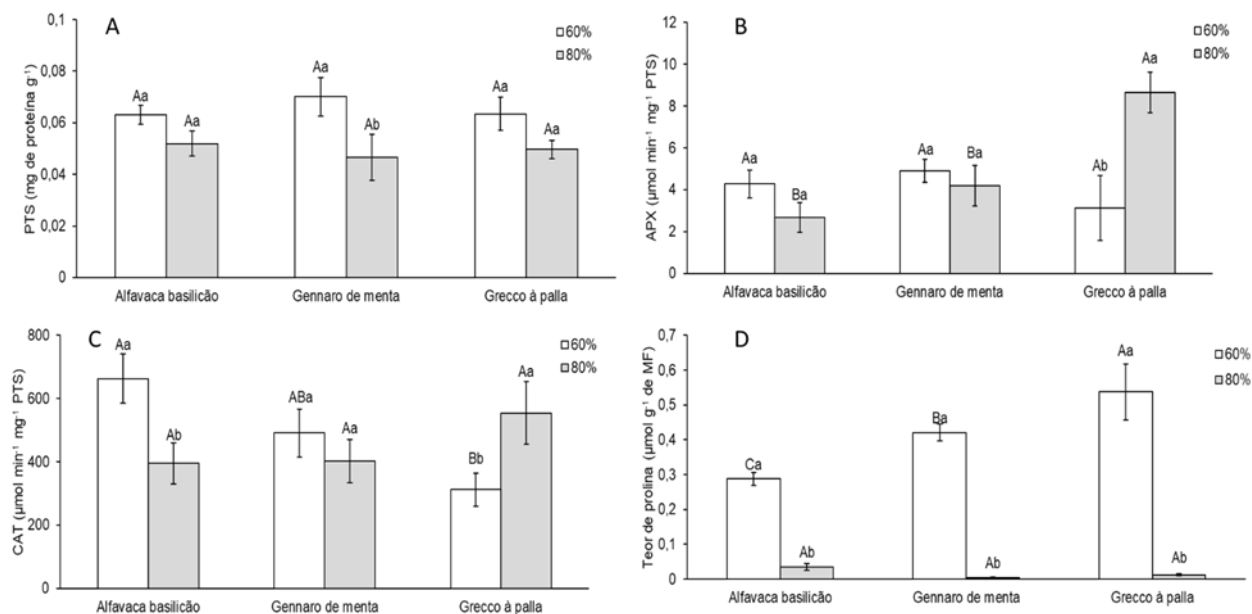


Figure No. 3

Total soluble protein content (A), peroxidase ascorbate (B), catalase (C) and proline content (D) in basil leaves at 75 DAP. Averages followed by the same letter do not differ statistically by the SNK test (5%). Uppercase letters compare the averages within the water levels, and lowercase letters compare the averages within the varieties

Essential oils

For the evaluation of essential oils, through the analysis of the main components, relationships or groupings were observed between the variables analyzed, and in this research the primary relationship refers to the investigation of 3 varieties of basil subjected to levels of 60 and 80% moisture in the soil (Figure No. 4). Thus, considering the main components 1 and 2, they explain 83.88% of the variance observed in the experiment. In addition, it is noted that eugenol and linalool were the most expressive compounds for *Alfavaca basilição* at the level of 80% water in the soil and for *Grecco à palla* under moderate stress.

Gas exchange

The net carbon assimilation rate (Figure No. 5A), on the second day after the imposition of the water deficit did not show significant interaction, while for the first day after the imposition of the deficit, the variety *Grecco à palla* showed lower value when submitted to 80% of the water level of the soil and, on the third day after, *Alfavaca basilição* showed a

reduction in the assimilation of CO₂ to 60% of the water level of the soil. As for stomatal conductance (Figure No. 5B), it is noted that the varieties showed a behavior similar to the net carbon assimilation rate on the first and third days after the imposition of moderate stress; on the second day, however, the variety *Grecco à palla* reduced its conductance when in a water deficit state, differing from the results observed for the net carbon assimilation rate.

The results obtained for the rate of leaf transpiration (Figure No. 5C) demonstrate that the variety *Grecco a pala* after the imposition of the water deficit presented a significant reduction in the condition of moderate stress on the first day, maintained the same rate on the second and presented an increase on the third. The *Alfavaca basilição* variety showed no significant difference from the control on the first day after stress was imposed, but decreased significantly over the days. For the efficiency of water use (Figure No. 5D), there was no significant interaction between the factors. *Grecco à palla* showed similar behavior on the first and third days after the imposition of the water deficit,

reducing its efficiency to the level of 80% of water in the soil. On the second day, no significant differences were found, and Alfavaca basilição demonstrated the

same efficiency in the use of water regardless of adverse conditions.

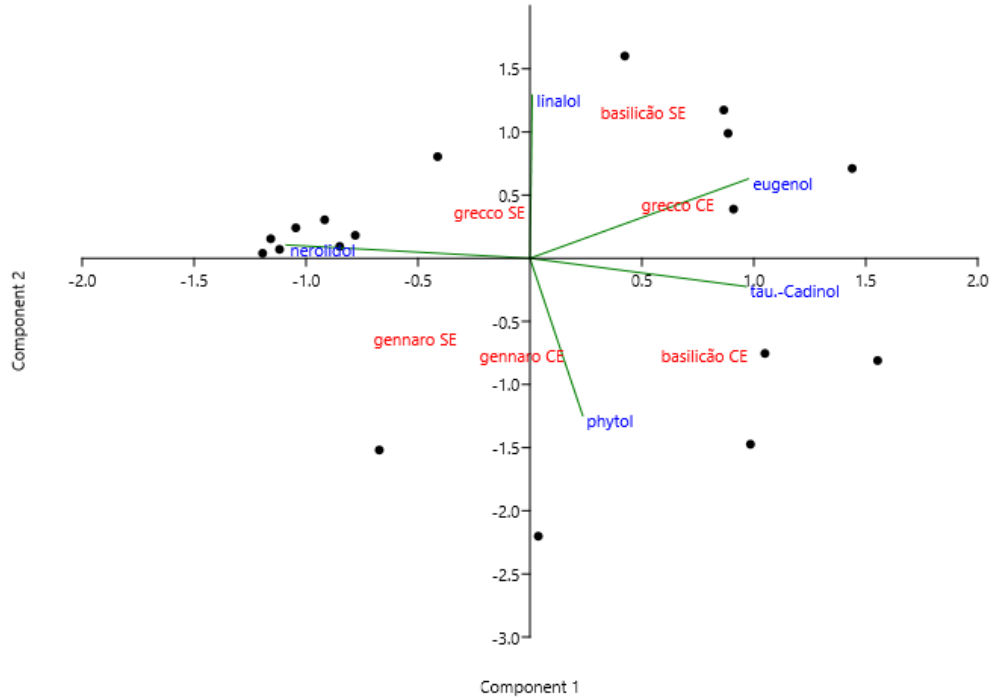


Figure No. 4

Biplot graph for basil varieties submitted to different levels of water in the soil: with stress (CE) or without stress (SE), in relation to the constitution of essential oils

Electrolyte leakage

The leakage of cellular electrolytes (Figure 6) was greater in plants subjected to water deficit, where damage was observed in the magnitudes, approximately: 29; 30 and 40% for Alfavaca basilição, Gennaro de menta and Grecco à palla, respectively, when compared to plants treated with 80% moisture in the soil.

DISCUSSION

It is considered that the plants showed completely different behavior during both phenological stages, but that in the joint analysis of the results these experiments highlight the ability of these plants to

resume their homeostasis, after being subjected to adverse conditions, demonstrating their characteristics of resilience and adaptation to the environment (Holling, 1973).

In the vegetative phase, all varieties, physiologically, showed good homeostatic behavior, as they managed to regulate their leaf water content (Figure No. 1), and the Gennaro de menta material reduced only by 8.1% under stress, and still managed to keep its RWC stable. The maintenance of homeostasis is directly correlated with the physiological stability of plants in the face of adverse conditions (Souza & Buckeridge, 2004; Camargo-Bortolin et al., 2008).

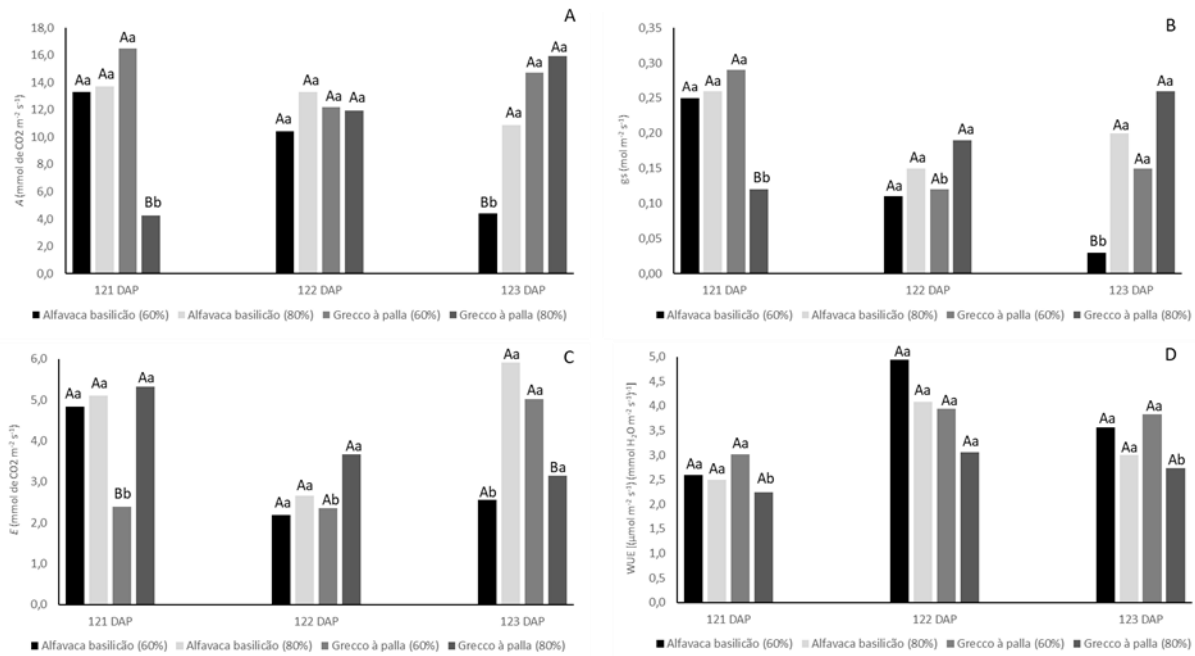


Figure No. 5

Net CO₂ assimilation rate (A, $\mu\text{mol de CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (A); stomatal conductance (gs, $\text{mol m}^{-2} \text{ s}^{-1}$) (B); leaf transpiration (E, $\mu\text{mol de CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (C) and water use efficiency (WUE, $[(\mu\text{mol m}^{-2} \text{ s}^{-1}) / (\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}) - 1]$) (D), in two varieties of basil after imposing the water deficit at 121, 122 and 123 DAP. Averages followed by the same letter do not differ statistically by the SNK test (5%). Uppercase letters compare the averages between the water levels, and lowercase letters compare the averages within the varieties

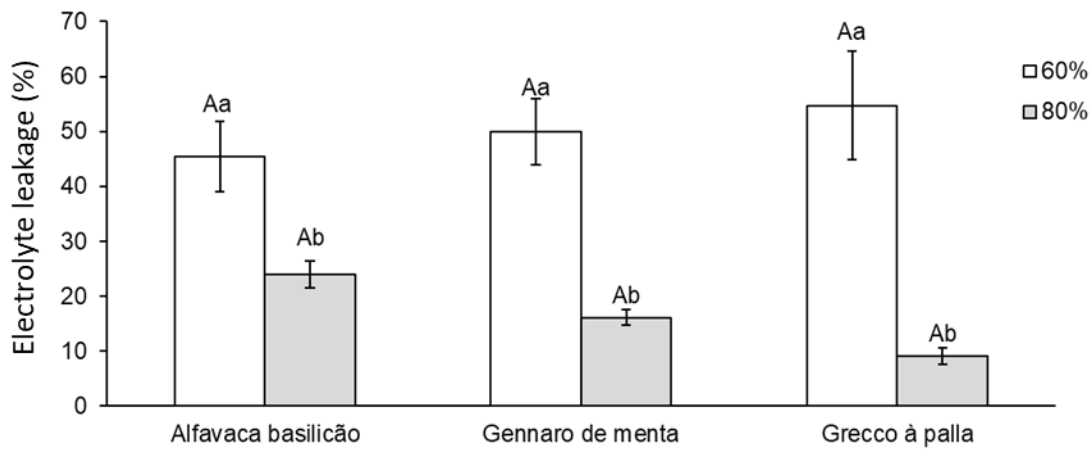


Figure No. 6

Extravasation of electrolytes from the leaves at 120 DAP. Averages followed by the same letter do not differ statistically by the SNK test (5%). Uppercase letters compare the averages within the water levels, and lowercase letters compare the averages within the varieties

In the flowering phase, the variety Grecco à palla suffered significant reductions in its leaf water content (Figure No. 1) when exposed to moderate stress, differing from the vegetative phase, indicating that the varieties were not able to maintain homeostasis in these conditions. This fact is evidence that, at this stage, these varieties have a strategy to escape water stress through morphophysiological changes (Galle *et al.*, 2011; Kloeke *et al.*, 2012), such as leaf fall and flower abortion, rather than the tolerance strategy. According to Santos and Carlesso (1998), when plants are subjected to water deficit at the beginning of their cycle, they are more easily able to adapt to the conditions imposed.

The results indicate that the lack of water reduces the cellular water status, however the Gennaro de menta material showed physiological aptitude to support the water deficit, these reductions in basil RWC due to water stress were also reported by Radácsi *et al.* (2010), who, studying different levels of field capacity (70, 50 and 30%), found that the condition of greater water stress resulted in a 20% decrease in RWC, compared to control treatment plants (which received water corresponding to 70% of the field capacity of the soil).

When exposed to 80% water, the three varieties showed an increase in the pigment content, or did not show variability if subjected to water reduction, as in the case of Alfavaca basilicão (Figure No. 2), this pattern of adjustment in chlorophyll content as a function of the water level available to plants has already been reported in the culture of *O. basilicum* L. (Khalid *et al.*, 2006),

The reduction in the levels of photosynthetic pigments (Figure No. 2) for the Gennaro de menta under conditions of moderate stress is an indication that the genotype has entered a state of water stress (Ashraf & Harris, 2013), where this reduction occurs due to oxidative damage (Egert & Tevini, 2002). Similar results were found by Khordi *et al.* (2013) when working only with a variety of basil under water deficit. Also, according to these authors, the reduction of chlorophyll in response to environmental stresses depends on the genotype of the plant, and this may have influenced the results of the present study, since three varieties of basil were used. Drought can reduce chlorophyll content in species not tolerant to water deficit (Corrêa *et al.*, 1987).

As for the content of total soluble proteins (TSP), the varieties obtained a behavior similar to the

RWC, observed in this research, where only the Genaro de menta reduced its amount to 80% of water (Figure No. 3). According to Ravi *et al.* (2020), non-structural carbohydrates and soluble proteins, can provide protection mechanisms against water stress, by inducing osmotic adjustment of the cell.

Considering the results found, it is possible to infer that these varieties regulated their metabolism in an adequate way to withstand the condition of moderate stress (60% of water level).

Evaluating the enzymatic antioxidant metabolism (Figure No. 3), it was observed that the variety Grecco à palla was sensitive under the level of 80% of water in the soil, since the increased activity of the APX enzyme directly reflects the increase in hydrogen peroxide, the substrate for inducing the enzymatic reaction (Shigeoka *et al.*, 2002; Cavalcanti *et al.*, 2007). The results for the activity of the CAT enzyme (Figure No. 3C) indicate that the Alfavaca basilicão, when compared to the other varieties, showed a higher production of reactive oxygen species when under moderate stress conditions, while the variety Grecco à palla showed greater enzymatic activity of CAT under stress-free conditions, which corroborates the results of the current research for the activity of the APX enzyme (Figure No. 3B). According to Greggains *et al.* (2000), these enzymes are an important primary defense against free radicals generated under stress conditions, being able to degrade H₂O₂ and result in water (H₂O) and molecular oxygen (O₂).

Grecco à palla was the variety that showed the highest accumulation of proline when compared to the others (Figure No. 3), which is an amino acid with an osmoprotective function when the plant is under water stress conditions (Bates *et al.*, 1973; Silva *et al.*, 2015; Moura *et al.*, 2016), which can give greater tolerance to the variety. The significant increase in proline content within all varieties in response to water deficit corroborates the results obtained by Khalid (2006), who observed that basil (*O. basilicum* L.) plants had a significant increase in proline content when subjected to water deficit. According to Mafakheri *et al.* (2011), this increase in proline content when the plant is subjected to stress is explained by the fact that this amino acid serves as a source of energy, nitrogen and carbon for tissue recovery.

Pirbalouti *et al.* (2017), also observed similar behavior for this variable, noting that the maximum

proline content in two basil species (*O. basilicum* and *O. ciliatum*) was obtained when the plants were subjected to water stress corresponding to 30% of the field capacity. Similarly, Khordi *et al.* (2013), observed that the levels of proline in basil increased significantly when the plants were subjected to soil moisture equivalent to 60 and 30% of the field capacity. These authors report that proline is a widely occurring amino acid in higher vegetables, and its accumulation in large quantities in plants is related to the occurrence of environmental stresses, such as drought and salt stress.

It is observed that the varieties showed a significant increase in proline concomitant to the higher percentages of electrolyte leakage. The leakage of electrolytes, according to Bajji *et al.* (2002), may be a response due to the limitation in photosynthesis, which results in membrane damage at the leaf level, being one of the first signs of stress.

As a defense mechanism (Ashraf & Foolad, 2007), there is an increase in the concentration of proline, which will act as an osmoregulatory agent.

For essential oils (Figure No. 4), there is an independent gradient, characterized by a positive grouping between tau-caudinol and phytol, while nerolidol has a negative behavior in relation to component 1. Linalool, on the other hand, component most found in basil plants (Radünz, 2004; Carvalho Filho *et al.*, 2006), was antagonistic to phytol, for component 2. These results are due to the distinct availability of water for cultivation, and the diversified metabolism that exists between the varieties of basil, in line with the results observed in this experiment.

It should be noted that, in the same quadrant, linalool and eugenol are identified as the most expressive oils for the *Alfavaca basilicão* varieties under moderate stress and *Grecco à palla* without stress. The behavior of medicinal plants, in terms of the production of compounds, such as alkaloids, flavonoids and essential oils, is directly influenced by the water deficit (Bortolo *et al.*, 2009), however, the level and duration of stress are crucial in the responses of these plants, in terms of quality and quantity (Alvarenga *et al.*, 2011).

The results obtained for gas exchange allow us to affirm that, for photosynthesis, two days of water deficit does not provide a condition of water stress for the studied varieties, while 3 days caused the reduction of CO₂ assimilation, stomatal conductance

and leaf transpiration for the *Alfavaca basilicão* genotype.

The reduction in net CO₂ assimilation after 3 days of water restriction (Figure No. 5) constitutes a physiological response to water deficiency, and concomitant to the reduction of the photosynthetic rate, there is a reduction in the photoassimilated drains (Bueno *et al.*, 2011), there was also a reduction in stomatal conductance (Figure No. 5) after three days of water deficit for *Alfavaca basilicão*. It is a response where the plant limits the conductance of gases in the leaves in order to preserve reduce its water loss, thus, consequently limiting its photosynthesis (Mutava *et al.*, 2011).

For Flexas *et al.* (2004), stomatal conductance above 0.2 mol m⁻² s⁻¹ represents a situation of good water availability and values below 0.1 mol m⁻² s⁻¹ indicate conditions of severe deficit, while values between these two extremes demonstrate a situation of moderate stress. This statement reinforces our results, where *Alfavaca basilicão* obtained values below 0.1 mol m⁻² s⁻¹ on the third day after the imposition of the water deficit, thus characterizing a situation of severe deficit. For leaf transpiration (Figure No. 5), it is noticed that as the soil water becomes scarce, the varieties begin to reduce their transpiration rate, in order to reduce water loss and save water available in the soil, concomitant to the stomata closure (Silva *et al.*, 2015).

As for the efficiency of water use (Figure No. 5), under moderate stress, there was no variation proportional to stomatal conductance for the varieties, which indicates that the water deficit may result in better photosynthetic efficiency. This variable is an excellent tool for analyzing water consumption by productivity in plants of medicinal and aromatic interest, and water levels do not necessarily interfere in the productive pattern of basil plants (Ekren *et al.*, 2012)

It is noticed that the *Alfavaca basilicão* presented the expected pattern for gas exchange, since, when in a situation of water stress the plant reduces its stomatal conductance, transpiration and, consequently, photosynthesis. In contrast, *Grecco à palla* showed inconclusive responses.

The results obtained in determining the leakage of electrolytes (Figure No. 6) demonstrated that it was higher in all varieties when subjected to the driest condition (60% of the water level), corroborating the results obtained by Khordi *et al.*

(2013), who concluded that the decrease in soil moisture from 100 to 30% resulted in an increase of 22% in the leakage of electrolytes in basil plants. According to Mahajan and Tuteja (2005), the increase in electrolyte leakage is one of the effects of water stress, which indicates dehydration of the protoplasm and can result in disorders in cellular metabolism.

Briefly, during the *Ocimum* development the plant physiology, biochemistry and metabolism are directly influenced by supply water for crop. In this sense, the irrigation management can contribute to *Ocimum* better qualitative attributes, according to the variability among cultivars, similar discussion was presented by Kalamartzis *et al.* (2020a), Kalamartzis *et al.* (2020b) e Nejatizadeh-Barandozi (2020). Basil varieties present distinct physiological and metabolic responses when subjected to water restriction, a behavior also observed in the different phenological stages, with the vegetative phase as the one with greater tolerance to water restriction.

CONCLUSIONS

The Alfavaca basilição variety was the most sensitive

to water restriction, in the evaluated parameters. The variety Grecco à palla showed greater tolerance to water deficit in the reproductive phase compared to the other varieties, suggesting that the increase in the proline amino acid is an osmoregulator, which contributes to preserve its photosynthetic capacity, and efficient use of water. The Gennaro de menta genotype showed greater sensitivity when exposed to conditions of good water availability. Among the five major metabolic factors evaluated, the most expressive for vegetative phase in the two water levels in the soil were linalool and eugenol, with little participation of nerolidol.

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