Biology

Putrescine and deficit irrigation as regulatory factors in basil plants metabolism and morpho-physiology

Putrescina e irrigação deficitária como fatores de sinalização do metabolismo e morfofisiologia de manjericão

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ABSTRACT

The purpose of this study was to alleviate the harmful effects of water restriction, by applying foliar doses of putrescine (0, 50, 100 and 150 mg L^{-1}) on basil plants, as well as, understand the different soil water levels (60% and 80% of soil capacity) on plant development. We evaluated the physiology and metabolic pathways of plants. Basil plants under water restriction (60% of soil capacity) reduced their growth and dry matter accumulation, leaf water status, physiological variables, and photosynthetic pigments. Furthermore, the exogenous putrescine sprays, influenced the biometric and physiological variables, photosynthetic pigments, and proline levels. Our research suggests that exogenous putrescine sprays promote different behaviours on *Basil* spp., through the activation of tolerance mechanisms in basil plants under moderate water deficit, principally by the proline accumulation in the leaves. Moreover, the oil chemical profile was determined by the water availability in the soil.

Keywords: Polyamine; Water available; Plant physiology; Plant metabolites

RESUMO

Este estudo propõe analisar o uso de doses crescentes de putrescina (0, 50, 100 and 150 mg L^{-1}), para aliviar efeitos prejudiciais do estresse hídrico em plantas de manjericão, bem como entender a ação de diferentes níveis de água no solo (60% e 80% capacidade de solo) sobre o desenvolvimento dessas plantas. Foram avaliadas vias fisiológicas e metabólicas das plantas de manjericão. Plantas de manjericão sob restrição hídrica (60% capacidade de campo) reduziram seu crescimento e acúmulo de

matéria seca, status hídrico foliar, variáveis fisiológicas e conteúdo de pigmentos fotossintéticos. Além disso, as pulverizações exógenas de putrescina influenciaram as variáveis biométricas e fisiológicas, os pigmentos fotossintéticos e os níveis de prolina. Nossa pesquisa sugere que pulverizações exógenas de putrescina promovem diferentes comportamentos em *Basil* spp., através da ativação de mecanismos de tolerância em plantas de manjericão sob moderado déficit hídrico, principalmente pelo acúmulo de prolina nas folhas. Além disso, o perfil químico do óleo foi determinado pela disponibilidade de água no solo.

Palavras-chave: Poliaminas; Disponibilidade hídrica; Fisiologia vegetal; Metabolitos

1 INTRODUCTION

Basil belongs to Lamiaceae family, encompassing over 7,200 species and 240 genres (Harley *et al*., 2004). These plants are rich in secondary metabolites having antioxidant, antifungal and bactericide properties (Koroch *et al*., 2007). Nevertheless, the biosynthesis of secondary compounds in basil plants diverges according to genotype, environment, and time of harvest (Scagel; Lee, 2012). and its profile can be modified by anthropic management, as deficit irrigation or fertilization (Castro *et al*., 2022; Pirbalouti *et al*., 2017), which contributes to increase the secondary compounds of interest in these plants. Also, some plant physiological processes are modified by anthropic management, such as net assimilation of carbon dioxide, stomata opening and closure, leaf expansion, vegetal growth, and secondary compounds synthesis (Mahajan *et al*., 2020).

In order to mitigate the negative effect of water scarcity, several nitrogen-containing compounds are accumulated in plant tissue in response to stresses, such as amino acids (proline, arginine), ammonium quaternary (glycine betaine) and polyamines (Parvaiz Ahmad, 2012). The polyamines are small aliphatic compounds present in plants, animals, and bacteria, which include spermidine, spermine; and its precursor putrescine. In addition, polyamines are compounds with the function of regulating the physiological processes of plant development (Mustafavi *et al*., 2018). Furthermore, they are involved in various metabolic processes in plants including gene expression, protein and DNA synthesis, cell division and differentiation, somatic

embryogenesis, organogenesis, dormancy breaking, flower and fruit development, and senescence (Shi; Chan, 2014; Tavladoraki *et al*., 2012). Proving the beneficial effect of exogenous application of polyamines on aromatic plants production and protection under water deficit (Abd Elbar *et al*., 2019).

Basil plant is an aromatic species susceptible to abiotic and biotic stresses. For this reason, to apply exogenous putrescine on basil plants subjected to water deficit seems as an alternative crop management to alleviate the negative effects of water restriction in morphophysiological characteristics and profile of essential oils. Therefore, this work aims to evaluate the effect of foliar application of putrescine on agronomic production, content, and quality of essential oils of basil plants subjected to different water levels.

2 MATERIAL AND METHODS

2.1 Plant materials and site description

Propagules of basil plants var. Lemon were obtained from mother-plants cultivated in a garden of medicinal plants, at the Universidade Federal de Viçosa - Campus Rio Paranaíba, (19º12'49"S and 46º13"57"W). Small segments (10 cm) cuttings were obtained from mother-plants branches, on February 22nd, these propagules were cultivated in seedlings tray (6cm x 6cm x 9cm) filled with oxisol (chart 1) and placed in a greenhouse, under shading, with soil well-watered and environment air temperature between 18 and 35 ºC, during 20 days.

Table – Soil chemical profile. Rio Paranaíba, MG. 2019

pH	Organic matter	P	K	S	Ca	Mg	Al	Zn	B	Cu	Fe	Mn	CEC	Base saturation
---	dag.dm^{-3}	mg.dm^{-3}			cmol.dm^{-3}			mg.dm^{-3}					cmol.dm^{-3}	%
5.5	2	4.2	26	9	2.1	0.7	0.3	0.5	0.2	0.9	34	3.8	7.7	36.9

Source: Authorship (2021)

There after, 24 plantlets were transplanted to plastic pots (8.5 dm^{-3}) filled with oxisol, having fertilization carried out twice, as follows: during the plant transplant we applied 5 g per pot of the fertilizer formulated: urea; single superphosphate and potassium chloride (10:10:10); and during vegetative growth at 18 DAT, 20 g per pot of same fertilizer were applied. Further crop practices followed the commercial farms recommendation. The first pruning occurred at 18 days after transplantation (DAT). All laboratory procedures were carried out in Crop Physiology and Metabolism Laboratory (LAFIMEPRO).

2.2 Experimental design and irrigation regime

The study was arranged as a factorial experiment based on a randomized complete block design (RCBD), with three replications. Treatments consisted of two factors (4 x 2). The first factor was the putrescine (Put) levels (0, 50, 100 and 150 mg L^{-1}) and the second factor was deficit irrigation (80% and 60% of soil capacity). Where the treatments were: T1= Put 0 mg L^{-1} + 80%, T2= Put 0 mg L^{-1} + 60%, T3= Put 50 mg L^{-1} + 80%, T4= Put 50 mg L^{-1} + 60%, T5= Put 100 mg L^{-1} + 80%, T6= Put 100 mg L^{-1} + 60%, T7= Put 150 mg L^{-1} + 80% and T8= Put 150 mg L^{-1} + 60%. The plants were pulverized three times with a 30 mL putrescine solution per plant, in each treatment, at 36, 43 and 60 DAT.

The irrigation regime was calculated through pot capacity, according to the equation:

$$\theta w = \frac{Wet\ soil\ mass\ (g) - Dry\ soil\ mass\ (g)}{Dry\ soil\ mass\ (g)}$$

where: θw = volumetric water content

Being considered 80% of pot capacity as adequate water level, and 60% as mild water deficit. After the last putrescine pulverization, the plants were submitted to the irrigation regimes according to the treatments previously described and kept for 10 days.

2.3 Biological and chemical analysis of basil plants

Two samplings were done at 55 and 60 DAT. In both days, relative water content (RWC), photosynthetic pigments, physiological variables and proline were measured. At 55 DAT, total soluble protein and antioxidant proteins were measured. At 67 DAT, height and plant dry matter were measured.

2.4 Biometric measures: height, fresh and dry matter

At 67 DAT the plant height was measured with a ruler, from the shoot base to the top. The shoot plant was weighed on a precision scale to measure its fresh matter (g), then all the materials were dried in an oven for 24h at 40 ºC to obtain the shoot dry matter (g).

2.5 Leaf relative water content (RWC), gas exchange, pigments, and enzymes activity

At 55 and 60 DAT, the RWC was measured, and calculated according the equation:

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} \; x \; 100 \tag{1}$$

where: RWC = leaf relative water content; FW = leaf fresh weight; DW = leaf dry weight; TW = leaf turgid weight.

The fully expanded leaf was collected, and fresh weight (FW) was obtained, after the same leaf was immersed in falcon tubes, containing 10 mL of distilled water, to obtain the turgid weight (TW). Then the samples were oven dried at 60 °C during 72 hours and weighed to determine the dry weight (DW).

Also, at 55 and 60 DAT, the leaves gas exchange measurements were taken, to get the CO_2 assimilation (A), stomatal conductance (gs), foliar transpiration (E), and intercellular CO_2 (Ci), and the intrinsic water use efficiency (WUE – A/E) and instantaneous carboxylation efficiency (A/Ci) were estimated. These procedures were

performed between 9:00 and 10:00am, through infrared gas analyzer (LI-6400XT; LI-COR Inc., Lincoln, NE, EUA), connected to the fluorometer (LCF-40 LI-COR Inc.) under photosynthetically active radiation (Q) fixed at 1,200 µmol m^{-2} s^{-1}, CO_2 at IRGA's chamber of 400 µmol mol^{-1} and air temperature of 25 °C.

Simultaneously to gas exchange analysis, full-expanded leaves have been cut (± 0.1g) and inserted into a plastic tube (50 mL) containing 10 mL of acetone 80% (v/v), without maceration (Macedo *et al.*, 2013), for quantification of chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), chlorophyll total (Chl total) (Witham *et al.*, 1971) and carotenoids (Car) (Lichtenthaler; Wellburn, 1983). All samples were measured in UV-Vis spectrophotometer, The results were expressed in milligrams of the pigment by a gram of fresh weight of the leaf tissue (mg g^{-1}).

$$Chlorophyll\ a = [(12{,}7\ x\ A_{663} - 2{,}69\ x\ A_{645})\ x\ \frac{V}{1000\ x\ W}$$

$$Chlorophyll\ b = [(22{,}9\ x\ A_{645} - 4{,}68\ x\ A_{663})\ x\ \frac{V}{1000\ x\ W}$$

$$Total\ chlorophyll = [(20{,}2\ x\ A_{645} + 8{,}02\ x\ A_{663})\ x\ \frac{V}{1000\ x\ W}$$

$$Carotenoids = \frac{[(1000\ x\ A_{470} - 3{,}27\ x\ Cl\ a - 104\ x\ Cl\ b)/229]}{1000\ x\ W}$$

where: A_{470} = absorbance at 470 nm; A_{645} = absorbance at 645 nm; A_{663} = absorbance at 663 nm; W = sample weight, V = acetone volume (80%)

The total soluble protein (TSP) was determined according to the methodology described by Bradford (Bradford, 1976), using bovine serum albumin (BSA) as standard curve.

For antioxidant enzymes activity, fresh leaves (± 200 mg) were grounded in mortar content liquid nitrogen, after ascorbate peroxidase (APX, EC 1.11.1.11) activity (µmol of APX min^{-1} mg^{-1} of TSP), catalase (CAT, EC 1.11.1.6) activity (µmol CAT min^{-1} mg^{-1} of TSP) and superoxide dismutase (SOD, EC 1.15.1.1) activity (U of SOD min^{-1} mg^{-1} of TSP) were evaluated (Sperotto, 2014). The proline content was quantified by the methodology proposed by Bates (Bates *et al.*, 1973).

2.6 Chemical analysis: essential oils

The essential oils were obtained through the hydrodistillation method with Clevenger apparatus. Basil plants were harvested and weighed. Then, fresh leaves were blended in 200 mL of water. The distillation was performed during 70 min. The obtained essential oils were weighed and stored in a dark place in order to be subsequently analyzed. Essential oils were analyzed by GC/MS using a Shimadzu QP2010. The identification of the constituents was assigned on basis of mass spectral comparison obtained from NIST library, visual interpretation and retention indexes comparison. Each constituent relative percentage was calculated through the rate between the area of each compound and the total area of the all-sample constituents.

2.7 Statistical analysis

The experiment was conducted under randomized complete block design (RCBD), in a greenhouse. Data were verified for normality and homogeneity of variances and then subjected to analysis of variance (ANOVA). Means were analyzed by ANOVA and further compared by the SNK test ($p<0.05$) using the R software. The graphs were plotted using Microsoft Excel® 2010.

3 RESULTS

After recognizing the normality and homoscedasticity of variances, the data were submitted to ANOVA, and the significant terms of the response variable were shown in Table S1 and S2.

3.1 Influence of water and putrescine on basil growth and development

The plant height did not have significant differences neither between water levels, 80% and 60%, nor putrescine concentrations (figure 1). However, plants with adequate water level (80%) had higher shoot height than those with mild water deficit

(60%). The concentration of 150 mg L^{-1} showed lower height values in both water availability levels. Dry matter had significant differences between putrescine doses in the 80% and 60% levels (figure 1). Plants belonging to 80% water level, had a higher dry matter accumulation in the 50 mg L^{-1} putrescine dose with 7.3 g of dry matter per plant, after that it decreased for higher doses. On the other hand, plants belonging to 60% water level, had a maximum dry matter accumulation in the 100 mg L^{-1} putrescine dose with 5.4 g of dry matter per plant.

Figure 1 – Plant height (A) and plant dry matter (B) of basil plants subjected to putrescine concentrations (0, 50, 100, 150 mg L^{-1}), under adequate irrigation (80%) and mild water deficit (60%). Values followed by the same capital letter do not show significant differences between water levels and lowercase letters between putrescine doses for the SNK 5% test. Vertical bars indicate the standard error of means (n = 3)
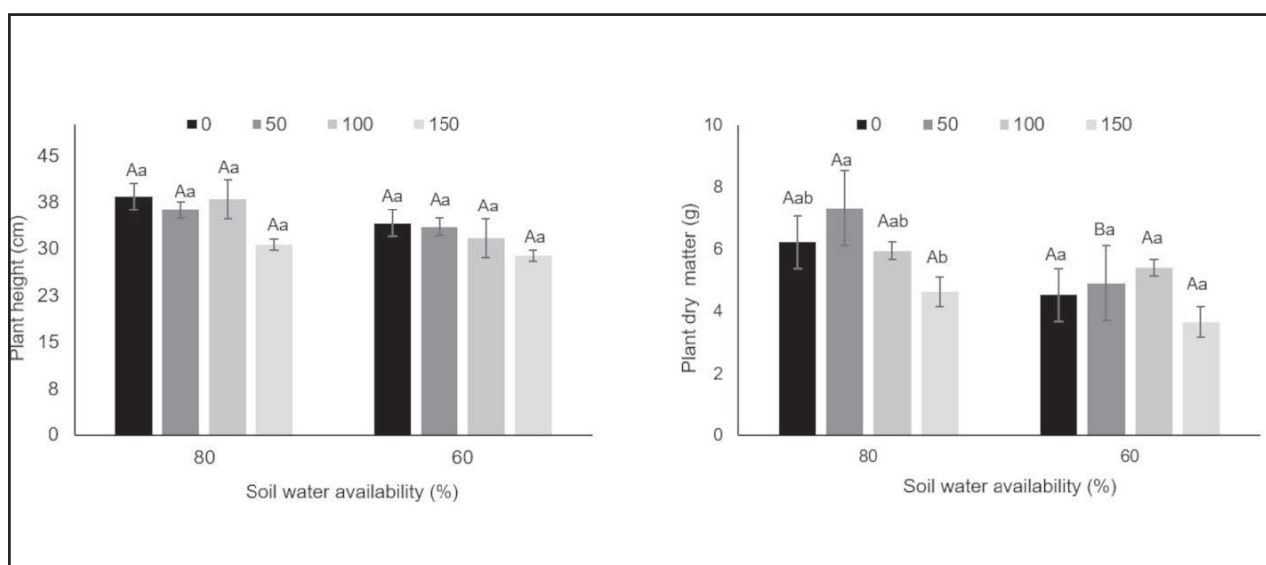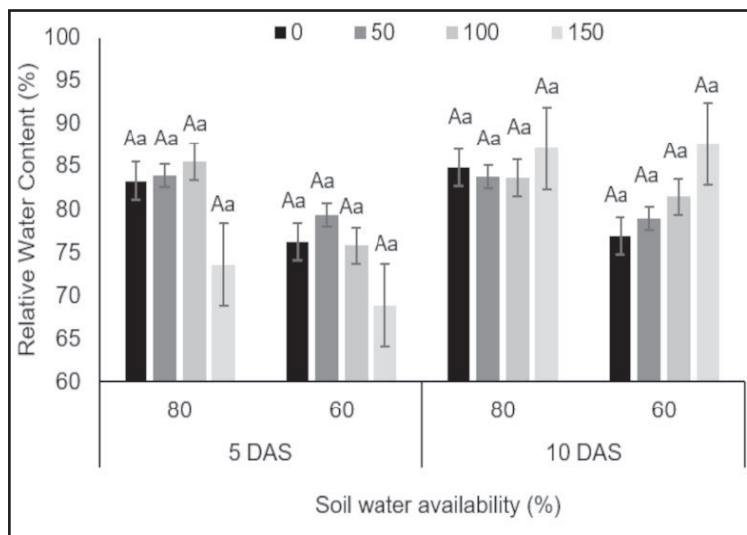

Source: Authorship

For RWC, at 5 and 10 days after stress (DAS), no significant differences were shown, neither between the two water levels, nor putrescine doses (figure2). However, at 5 DAS, plants under adequate irrigation had an increased tendency of the RWC values according to a higher putrescine dose applied until 100 mg L^{-1}. After that the RWC decreased. In plants under mild water deficit, the highest RWC was found in the

dose of 50 mg L^{-1}. Then, at 10 DAS, plants under adequate irrigation treated with 150 mg L^{-1} of putrescine, showed the highest RWC. In plants under mild water deficit, the RWC increased when the putrescines doses were higher.

Figure 2 – Relative water content (%) in basil plants subjected to putrescine concentrations (0, 50, 100, 150 mg L^{-1}), under adequate irrigation (80%) and mild water deficit (60%). Values followed by the same capital letter do not show significant differences between water levels and lowercase letters between putrescine doses for the SNK 5% test. Vertical bars indicate the standard error of means (n = 3)

Source: Authorship

The CO$_2$ assimilation had significant differences between the treatments for both sampling days (figure 3A). At 5 DAS, control plants and those ones sprayed with 50 mg L^{-1} of putrescine under adequate irrigation showed the highest CO$_2$ assimilation values. Plants under mild water stress did not have significant differences. However, control plants and those sprayed with 100 mg L^{-1} showed the highest CO$_2$ assimilation values. On the other hand, at 10 DAS, plants under adequate irrigation had higher CO$_2$ assimilation values for 100, followed by 50 and 150 mg L^{-1} of putrescine, respectively. Finally, for plants under moderate water deficit, 50 and 100 mg L^{-1} of putrescine had higher values of CO$_2$ assimilation.

Figure 3 – Net of CO_2 assimilation (A, A), stomatal conductance (gs, B), intercellular CO_2 (Ci, C), foliar transpiration (E, D), water use efficiency (WUE, E) and instantaneous carboxylation efficiency (A/Ci, F) in basil plants subjected to putrescine concentrations (0, 50, 100, 150 mg L^{-1}) under adequate irrigation (80%) and mild water deficit (60%) at 5 and 10 DAS. Values followed by the same capital letter do not show significant differences between water levels and lowercase letters between putrescine doses for the SNK 5% test. Vertical bars indicate the standard error of means (n = 3)
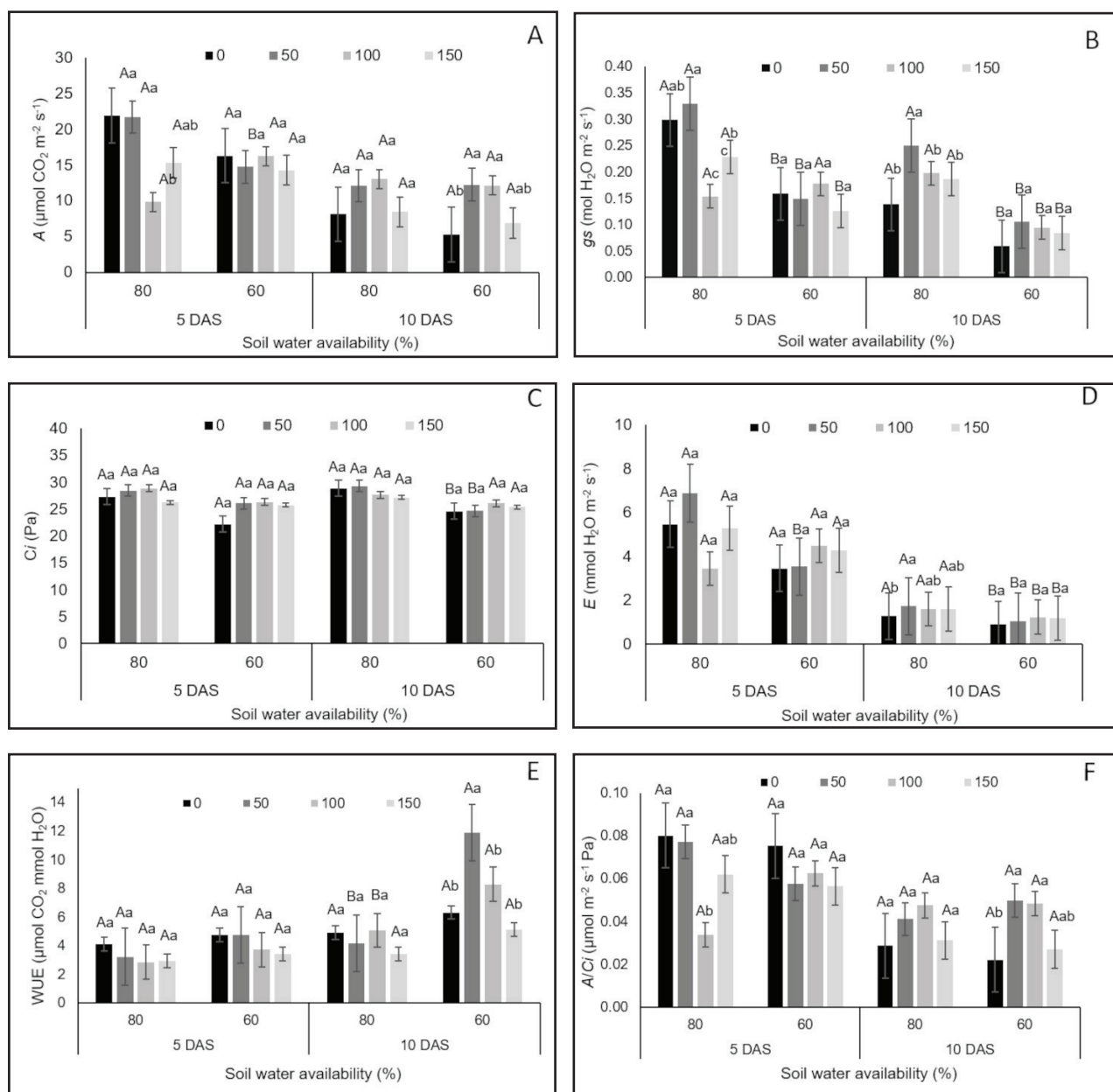
Source: Authorship

Concerning the stomatal conductance, at 5 and 10 DAS, it was possible to verify

significant differences between the treatments belonging to the 80% water level. Whereas plants irrigated with 60% water level had lower values than plants irrigated adequately (figure 3B). At 5 DAS, the highest stomatal conductance values were reached for the plants sprayed with 50 mg L^{-1} of putrescine, followed by the control plants and those sprayed with 150 and 100 mg L^{-1} of putrescine, respectively. Moreover, plants under mild water stress did not show significant differences between putrescine doses. However, plants sprayed with 100 mg L^{-1} of putrescine had higher stomatal conductance than the other putrescine concentrations within the same water level.

At 10 DAS, plants under adequate irrigation and sprayed with 50 mg L^{-1} of putrescine had higher significant values of stomatal conductance rather than the other treatments at the same water level. Yet, plants under mild water deficit did not have significant differences between the putrescine doses. However, the plants sprayed with 50 and 100 mg L^{-1} had higher stomatal conductance values than the other two polyamine doses.

For intercellular CO_2, at 5 and 10 DAS, there were no significant differences between the putrescine doses (figure 3C) and there were only differences between the water levels at 10 DAS. Plants under mild water deficit and sprayed with 0 and 50 mg L^{-1} of putrescine had significant lower values than the same doses in the plants with adequate irrigation. At 5 DAS, both water levels had a proportional increase of intercellular CO_2 with the putrescine doses until they reached 100 mg L^{-1}. Then, at 10 DAS, plants without water deficit and sprayed with 50 mg L^{-1} of putrescine, showed higher intercellular CO_2 values. Finally, plants under mild water deficit had a proportional increased tendency of intercellular CO_2 with the putrescine doses; until 100 mg L^{-1} of putrescine.

Foliar transpiration, at 10 DAS, just had significant differences between either the water levels or putrescine doses (figure 3D). At 5 and 10 DAS, plants under adequate irrigation showed higher foliar transpiration values than the plants under mild water deficit. Then, plants without deficit water and sprayed with 50 mg L^{-1} of putrescine

had higher foliar transpiration values than the other putrescine concentrations at the same water level. On the other hand, plants under mild water deficit and sprayed with 100 mg L^{-1} of putrescine had higher foliar transpiration values than the other concentrations.

Water use efficiency (WUE) had significant differences at 10 DAS between either the water levels or putrescine doses (figure 3E). At 5 DAS, plants under mild water deficit showed higher WUE values than the plants without water restriction. Furthermore, in both groups, plants sprayed with 0 and 50 mg L^{-1} of putrescine had higher WUE values than the other concentrations. At 10 DAS, plants under mild water deficit and sprayed with 50 and 100 mg L^{-1} of putrescine had higher WUE values than the plants under adequate irrigation.

Instantaneous carboxylation efficiency had significant effect for putrescine doses in plants under adequate irrigation and mild water deficit at 5 and 10 DAS, respectively (figure 3F). At 5 DAS, plants under adequate irrigation and sprayed with 0 and 50 mg L^{-1} had higher instantaneous carboxylation efficiency values than the plants under mild water deficit. Then, at 10 DAS, both groups of plants sprayed with 50 and 10 mg L^{-1} of putrescine had higher instantaneous carboxylation efficiency than the other putrescine levels.

The leaf content of Chl *a*, at 5 and 10 DAS, had significant differences of both water levels and putrescine doses (figure 4A). Also, at 10 DAS, plants of both water levels decreased their photosynthetic pigments content in comparison with the first sampling day. At 5 DAS, plants under adequate irrigation and sprayed with 50 mg L^{-1} of putrescine had significantly higher Chl *a* content than the other putrescine doses. Whereas plants under mild water deficit did not show Chl *a* significant difference between the doses, plants sprayed with 100 mg L^{-1} of putrescine had the highest Chl *a* content even compared with the same dose in plants under adequate irrigation.

Figure 4 – Chlorophyll a (A), Chlorophyll b (B), Chlorophyll total (C) and carotenoids (D)

in basil plants submitted to putrescine concentrations (0, 50, 100, 150 mg L^{-1}) under adequate irrigation (80%) and mild water deficit (60%) at 5 and 10 DAS. Values followed by the same capital letter do not show significant differences between water levels and lowercase letters between putrescine doses for the SNK 5% test. Vertical bars indicate the standard error of means (n = 3)
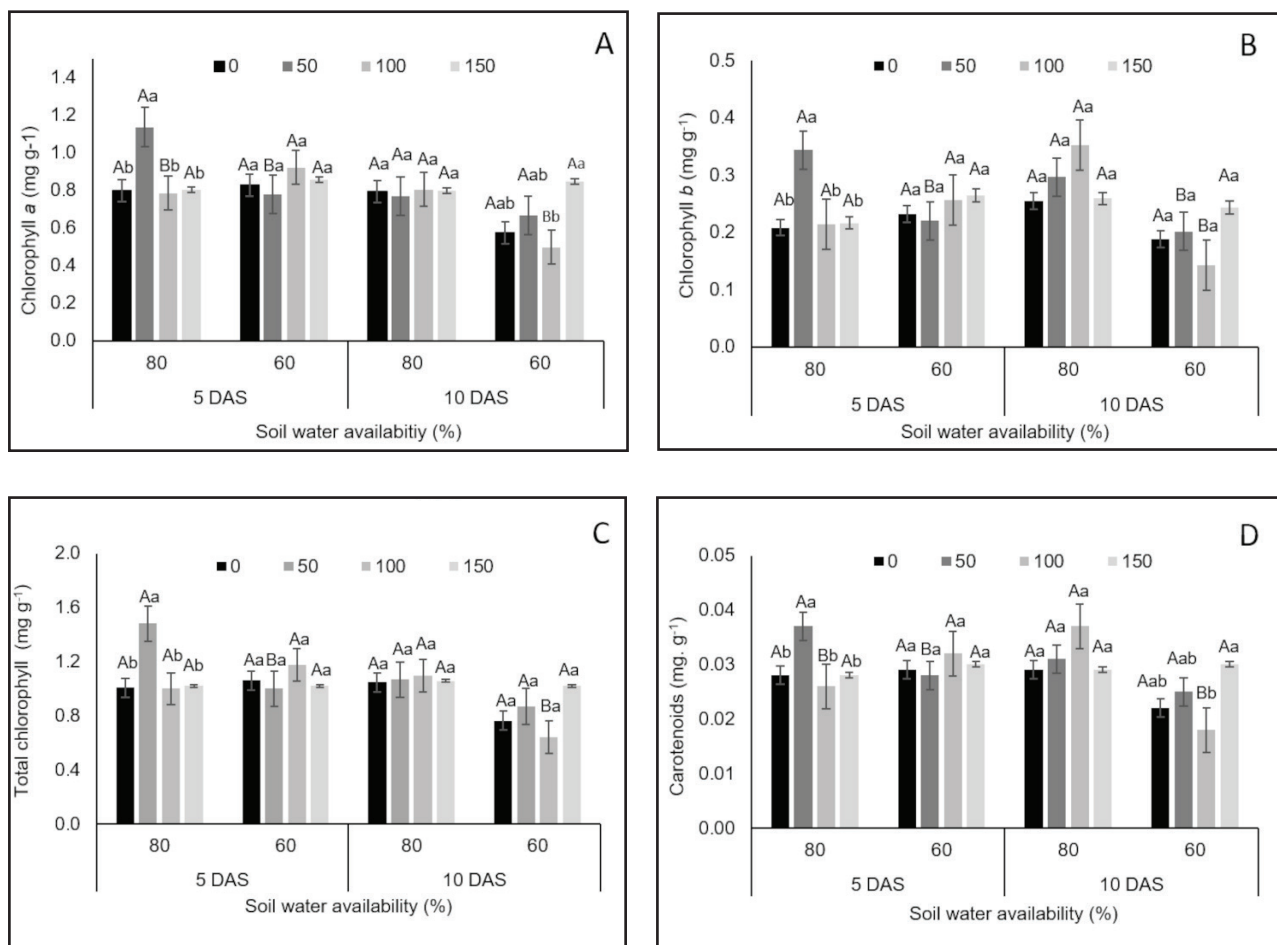
Plants under mild water deficit, at 10 DAS, only showed significant differences between the putrescine dose; plants sprayed with 150 mg L^{-1} had the highest Chl a content, followed by 50, 0 and 100 mg L^{-1} of putrescine. Only at 5 DAS, Chl b and Chl total showed significant differences of both water levels or putrescine doses. In contrast, at 10 DAS, Cl b and Cl total just had significant differences between water levels (figure 4B and 4C). At 5 DAS, plants under adequate irrigation and sprayed with 50 mg L^{-1} of putrescine had higher significant Chl b contents than the other putrescine

doses. On the other hand, plants under mild water deficit and sprayed with 100 and 150 mg L^{-1} of putrescine showed higher Chl *b* contents than the other concentrations.

At 10 DAS, plants under mild water deficit showed lower Chl *b* contents than the plants under adequate irrigation. The highest Chl *b* contents were displayed by the plants sprayed with 100 and 150 mg L^{-1} of putrescine for the 80% and 60%

water levels, respectively. At 5 DAS, plants under adequate irrigation and sprayed with 50 mg L^{-1} of putrescine had higher significant Chl total contents than the other putrescine doses. Then, plants under mild water deficit had a Cl total content 11% higher than the other putrescine doses. Finally, at 10 DAS, plants under adequate irrigation had the highest Cl total contents.

Carotenoids contents had a factor interaction in both sampling days. The putrescine doses effect was observed at 5 and 10 DAS in plants of 80% and 60% water levels, respectively (figure 4D). At 5 DAS, plants under adequate irrigation and sprayed 50 mg L^{-1} of putrescine had significantly higher carotenoid contents than the other putrescine doses in the same water level. Moreover, plants under mild water deficit and sprayed with 100 mg L^{-1} had a slight increase of 10% in the carotenoid contents in comparison with the other putrescine doses. Finally, at 10 DAS, plants under mild water deficit and sprayed with 150 mg L^{-1} of putrescine had the highest carotenoid contents.

Plants under adequate irrigation had higher total soluble proteins values than the plants under mild deficit water. Plants of 80% water level and sprayed with 50 mg L^{-1} of putrescine showed significant differences. However, plants of 60% water level did not have significant differences.

3.2 Influence of water and putrescine on basil antioxidant apparatus

For antioxidant metabolism: SOD, APX and CAT activity few significant differences were observed between treatments. Plants under adequate irrigation (80%) and subjected to 50 mg L^{-1} of putrescine showed higher APX activity when compared to deficit irrigation (60%) and subjected to 50 mg L^{-1} of putrescine (figure 5C). Moreover,

the highest CAT activity was performed by plants sprayed with 50 mg L^{-1} of putrescine, under 60% of soil water available (figure 5D).

Figure 5 – Total soluble protein (TSP, A), superoxide dismutase (SOD, B), catalase (CAT, C) and ascorbate peroxidase (APX, D) in basil plants subjected to putrescine concentrations (0, 50, 100, 150 mg L^{-1}) under adequate irrigation (80%) and mild water deficit (60%). Values followed by the same capital letter do not show significant differences between water levels and lowercase letters between putrescine doses for the SNK 5% test. Vertical bars indicate the standard error of means (n = 3)
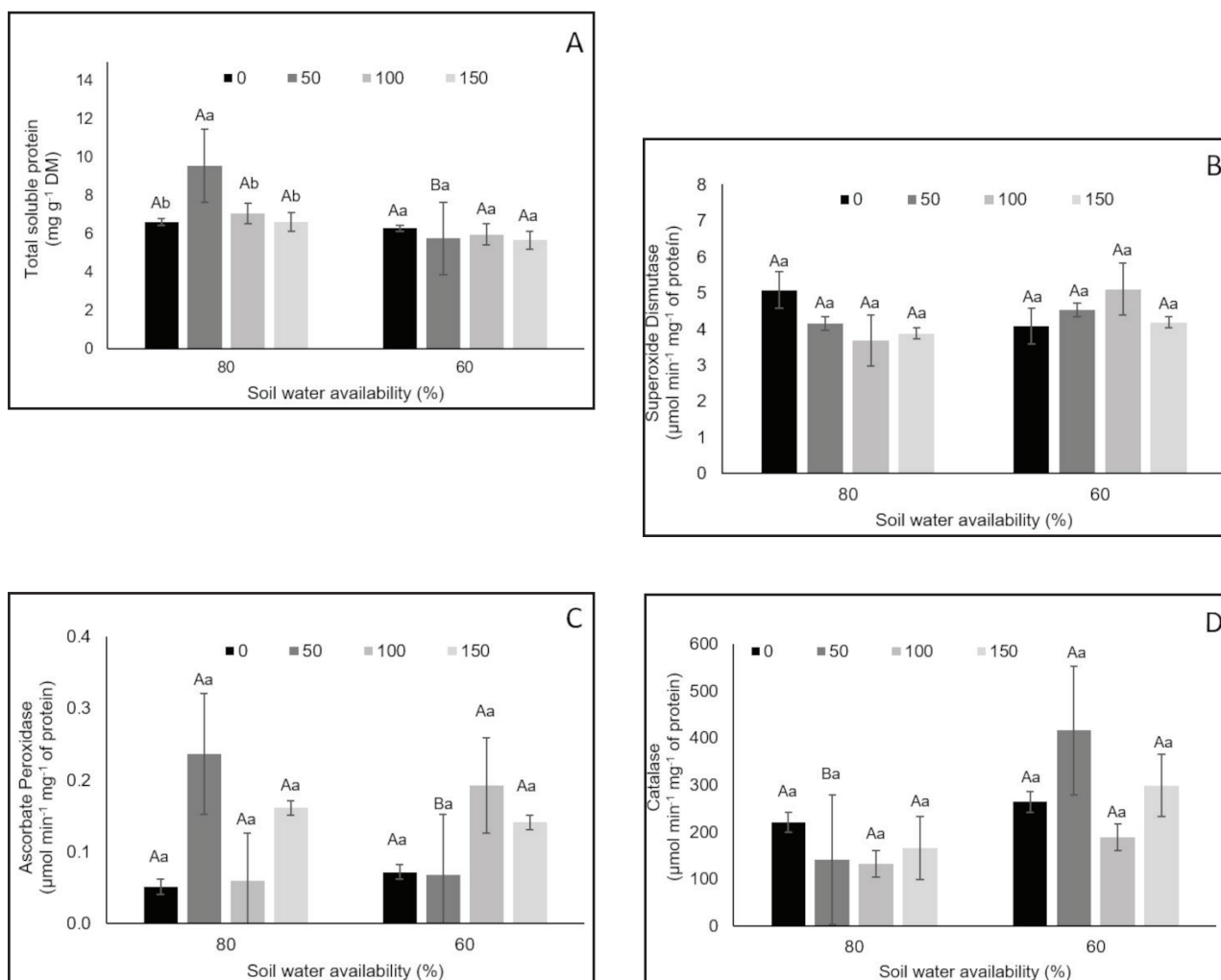
At 5 DAS, Proline content was relatively lower than the 10 DAS. At 10 DAS, just plants under mild deficit water had significant differences between putrescine doses

(figure 6), especially plants sprayed with 100 mg L^{-1} of putrescine. Regarding proline leaves content, at 5 DAS, we observed means lower than the 10 DAS. At 10 DAS, just plants under mild deficit water had significant differences between putrescine doses (figure 6), especially plants sprayed with 100 mg L^{-1} of putrescine.

Figure 6 – Leaf proline content in basil plants subjected to putrescine concentrations (0, 50, 100, 150 mg L^{-1}) under adequate irrigation (80%) and mild water deficit (60%) at 5 and 10 DAS. Values followed by the same capital letter do not show significant differences between water levels and lowercase letters between putrescine doses for the SNK 5% test. Vertical bars indicate the standard error of means (n = 3)
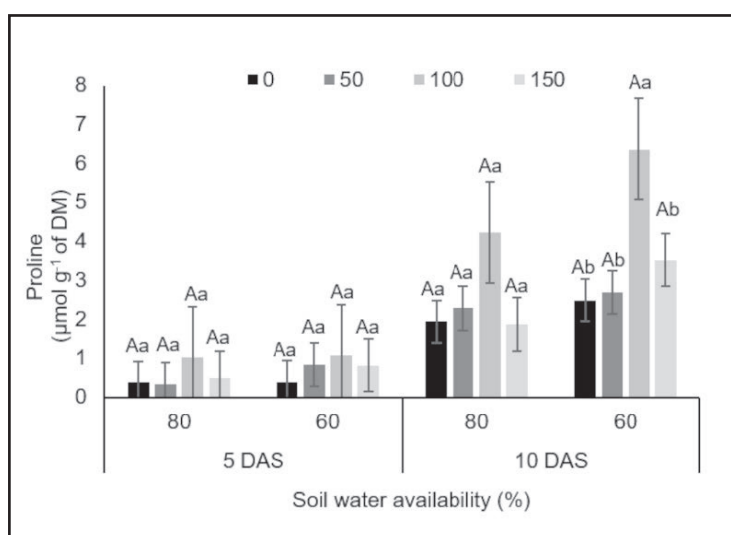
Source: Authorship (2021)

3.3 Influence of water and putrescine on basil essential oil

Secondary metabolites did not show significant differences between the putrescine doses. However, those had differences in intensity and components between the water levels in the soil (figure 7).

According to the VIP scores, essential oils show differences in the intensity and molecule type found for putrescine doses and water levels in the soil. Linalool and α-bulnesene were the main metabolites found in plants without putrescine application

and submitted to mild water deficit. On the other hand, the main metabolite was 2-acetoxy-1,8-cineole in plants with adequate irrigation. Plants sprayed with 50 mg L^{-1} of putrescine showed Isobornyl acetate in plants without water deficit, and α-muurolen and z-farnesene in plants with mild water deficit. Finally, plants sprayed with 100 mg L^{-1} of putrescine showed linalool and *cis*-3-hepten-1-ol without water deficit, and 2-Acetoxy-1,8-cineole in plants with mild water deficit.

Figure 7 – Main components (left) and VIP scores (right) for the PSL-DA analysis. Full filled squares in the right show metabolite intensity in basil plants sprayed with 0 (A and B), 50 (C and D) and 100 (E and F) mg L^{-1} of putrescine in adequate irrigation (a1, a3 and a5) and mild water deficit (a2, a4 and a6). Vertical bars indicate the standard error of means (n = 3)
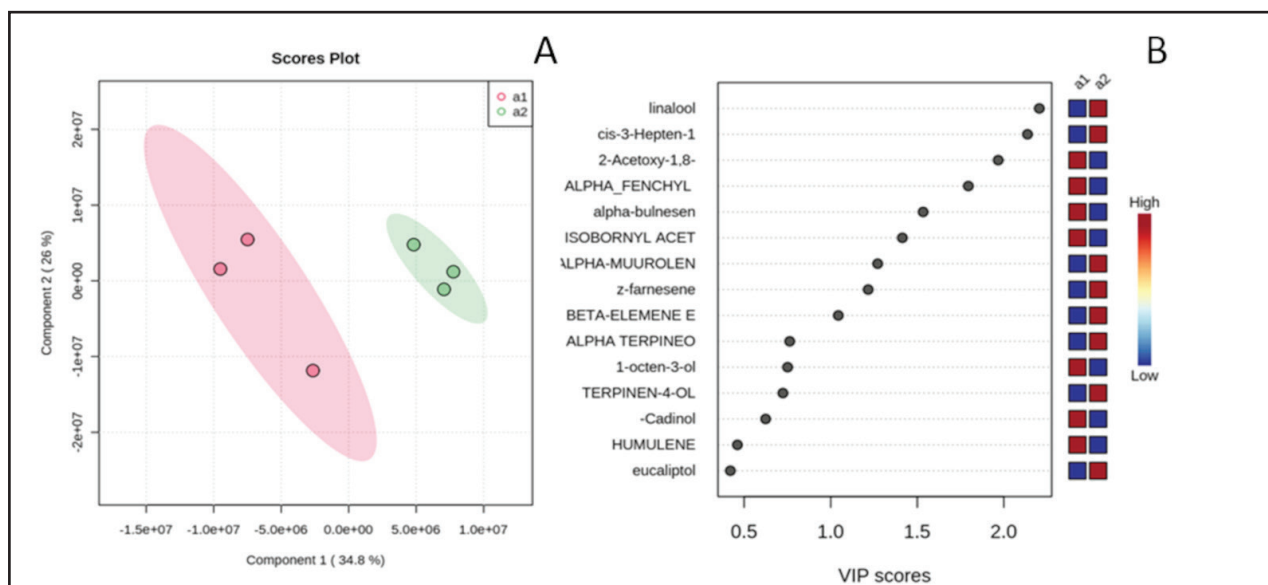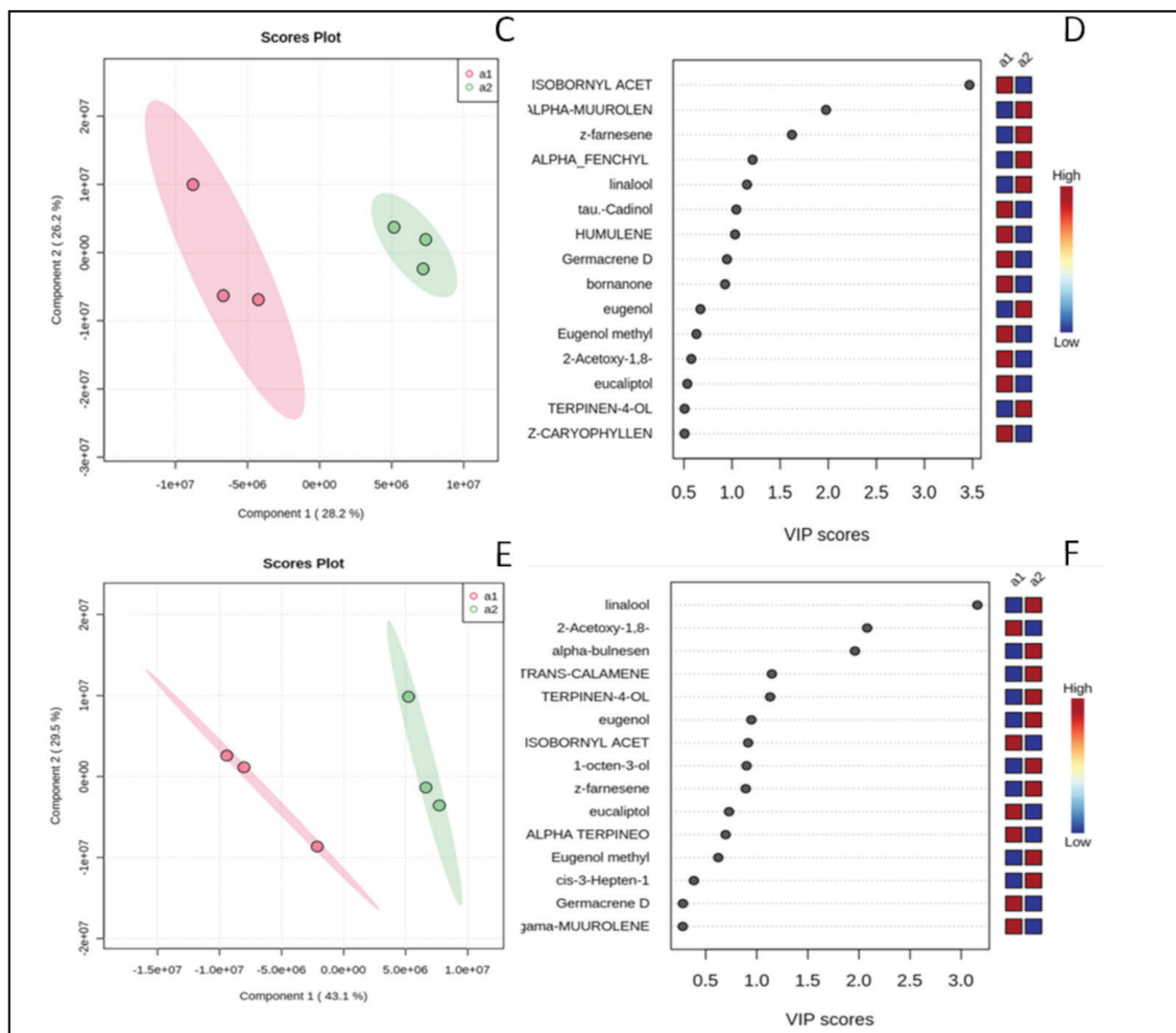
Figure 7 – Main components (left) and VIP scores (right) for the PSL-DA analysis. Full filled squares in the right show metabolite intensity in basil plants sprayed with 0 (A

and B), 50 (C and D) and 100 (E and F) mg L^{-1} of putrescine in adequate irrigation (a1, a3 and a5) and mild water deficit (a2, a4 and a6). Vertical bars indicate the standard error of means (n = 3)

(Conclusion)

4 DISCUSSION

Water restriction has a direct effect on physiological and structural processes in and potential damage in membranes, leading to increased solute concentrations inside the cells, for cell protection (Mahajan *et al*., 2020). In our research, plants under mild water deficit had a decrease of 33% in dry matter, mainly affecting cellular

expansion, stomatal conductance, photosynthetic yield, carbon structures production and its translocation to the principal growth sinks.

Our results showed that the plants without deficit irrigation and subjected to 50 mg L^{-1} of putrescine had the highest shoot dry matter accumulation. This behavior was led by the lower putrescine doses that increased the CO_2 assimilation at the 5 DAS. At 10 DAS, plants under mild water deficit and sprayed with 50 e 100 mg L^{-1} of putrescine had higher photosynthetic rates than the other treatments; therefore, higher putrescine doses lead to a negative effect on basil plants without water deficit and putrescine pulverization have a positive effect when the basil plant is suffering from a mild water deficit, and in periods of longer stress. Similar results of putrescine benefits were reported in basil (Talaat; Balbaa, 2010).

Plants sprayed with foliar putrescine have shown an alleviating mechanism against negative drought effects through well-regulated metabolic routes (Chen *et al*., 2019), the polyamines are plant growth regulators that can increase cellular division and link to the membranes in order to stabilize and protect them against peroxidation (Abbasi *et al*., 2017).

Keeping the water status in the plants cells is one of the challenges when they face a low availability of water in the root system, our results showed that the putrescine had an influence on the water leaves status adjustments. Basil plants submitted to mild water deficit (60% PC) reached higher RWC than 75%, however, both sample days did not show statistically significant differences between water levels and putrescine doses (figure 2). However, basil plants reflect a water consumption enhancement under mild water deficit related to a low transpiration (figure 3D) and a better adjustment of water use efficiency (figure 3E).

The similar water status in both groups of plants could be explained by the hydrophilicity which is the polyamines feature that plays a pivotal role in keeping the cellular turgor through the osmolytes accumulation in the leaves (Kuznetso; Svyakova, 2007). In fact, the highest proline contents were found in plants under mild water

deficit and sprayed with 100 mg L^{-1} of putrescine (figure 6) at 10 DAS.

Either polyamines or proline have the glutamate as a common precursor, then any change in their concentrations could modify the biosynthetic route of the other compound (PÁL *et al.*, 2018). Therefore, the mild water deficit could lead to using the available glutamate for the proline synthesis in the leaves, which is a biochemical signal against abiotic stress (Abdul Jaleel *et al.*, 2007). Proline synthesis helps the plants tolerate water deficit through the osmotic adjustments and membranes protection against the reactive oxygen species (ROS`s) action (Cramer *et al.*, 2011).

Perhaps proline had a special action in osmotic adjustment in the plant cells under mild water deficit, a fact that had a relation with similar RWC values even with the basil plants without deficit restriction. This behavior had a relation with the putrescine dose of 100 mg L^{-1} in plants under mild water deficit at 10 DAS (figure 6).

Dry matter accumulation was affected by water deficit, basil plants sprayed with putrescine could mitigate negative effects, especially when the water restriction is prolonged, it is made by the solute accumulation in the leaves. Hassan et al (Hassan *et al.*, 2018) reported that putrescine pulverizations improved some parameters such as: plant growth, RWC, chlorophyll contents and stomatic conductance in *Rosa damascena* under water deficit.

Understanding the relation between the duration and intensity of water restriction and external factors could help to unveil the different responses of the plants to the water deficit (Farooq *et al.*, 2009). Then, the duration and intensity of the water restriction had a small effect in the water status regulation in the leaves, however, there was a representative response between water availability and putrescine concentration in the dry matter accumulation at the end of the experiment.

Chlorophylls had a pivotal role in the photosynthetic mechanism and dry matter accumulation (Rhie *et al.*, 2014). Our results showed that water deficit conditions led to chlorophylls and carotenoids degradation. In contrast, basil plants well irrigated had an increase tendency in chlorophylls contents, except for the plants sprayed with 50 mg

L^{-1} of putrescine. In fact, Cl total contents were higher in plants with adequate irrigation than the plants submitted to mild water deficit, and it could be explained because ROS's tend to accumulate and promote the photosynthetic degradation in plants under water deficit (Ashraf, Harris, 2013), similar results were reported basil plants (Damalas, 2019). At 5 DAS, Chl *a*, *b* and total did not have differences between the water levels, however at 10 DAS, those contents were slightly higher in plants irrigated adequately. A possible adaptable strategy in a restricted environment could reduce the internal damage through the fewer solar radiation interception as a defense mechanism that will have an influence in the photosynthetic rate and dry matter accumulation.

Doses of 50 and 100 mg L^{-1} of putrescine had a positive effect in keeping higher Chl *a*, *b*, total and carotenoid contents in plants with 80% and 60% of PC, respectively. Some researchers have reported the photosynthetic pigments increase when the polyamines were used. Amri et al. (Amri *et al*., 2011) explained the thylakoids membranes protection especially in the protein-chlorophyll complex where the polyamines link and have a protecting effect of the photosynthetic apparatus (Abd Elbar *et al*., 2019).

Putrescine, chlorophyll and proline synthesis have the glutamate as a common precursor (Mohammadi *et al*., 2016), so we believe that a possible chlorophyll degradation would cause metabolic protective responses such as the raise of proline contents as it was observed in plants submitted to mild water deficit, especially at 10 DAS.

In our study, basil plants with 60% of PC had lower chlorophyll contents and dry matter accumulation than plants with 80% of PC. However, when those were pulverized with 100 mg L^{-1} of putrescine, the negative effect of the water restriction could be mitigated in a short term as we found at 5 DAS. It could be explained because polyamines could have a protection and keep the thylakoids membranes stability. In basil plants without any water restriction, glutamate and putrescine could be redirected for the chlorophyll synthesis and cellular division, respectively. On the other hand, carotenoid contents showed significantly lower values when the plants were

pulverized with 100 mg L^{-1} of putrescine in both water levels, potentially because of its function in photosynthetic processes and preventive to oxidative damage in cellular membranes (Takahashi; Badger, 2011).

Plants close their stomata as a first protective defense against the water deficit, which is modulated by abscisic acid increased concentrations (Bharath *et al*., 2021). Similar behavior was shown in this study, plants under mild water deficit had lower stomatal conductance (figure 3B) at 5 and 10 DAS than the plants with adequate irrigation. Stomatal closure and opening are regulated by many signaling mechanisms related to the water status either in the soil or in the leaf (Mahajan *et al*., 2020). One of the mechanisms is controlled by abscisic acid concentrations that make a decreased stomatal conductance (Waterland *et al*., 2010). In our study, the RWC values were over 70% (even in plants under mild water deficit at 10 DAS). So, the water status in the leaves could be regulated by the osmolyte adjustments. It may have happened because of the synthesis and accumulation of osmolytes in the leaves.

There will be greater restriction of leaf transpiration and a potential increase in water use efficiency (WUE) as a result of the stomatal closure (Ebeed *et al*., 2017). Moreover, there is a high reduction in CO_2 diffusion across the leaf mesophyll that leads to releasing high quantities of electrons and ROS's synthesis (Farooq *et al*., 2009). In those terms, the leaf transpiration was reduced by the stomata closure in plants under mild water deficit with higher effect at 10 DAS. So, this behavior was related to the duration and intensity stress imposed. A non-existence of significant differences between C*i* at 5 and 10 DAS, for both water levels, could be interpreted in two different scenarios. Firstly, plants submitted to mild water deficit tend to accumulate CO_2 in the leaf mesophyll during the early hours, when the stomata are still open. After that, the radiation reached the leaves and stomata closed, so CO_2 gets stuck in the intercellular spaces at the leaf mesophyll. On the other hand, plants well irrigated had a higher stomatal conductance, a fact that indicates the stomata were opened and allowed the CO_2 flux coming from the atmosphere.

At 5 DAS, plants under mild water deficit had a lower CO_2 assimilation than the plants watered adequately and sprayed with 50 mg L^{-1} of putrescine. At 10 DAS, plants without water deficit had the highest CO_2 assimilation, however, the difference was slight; and 50 and 100 mg L^{-1} of putrescine doses had higher CO_2 assimilation and instantaneous carboxylation efficiency (A/Ci) than the other polyamine doses. This behavior is probably related to the polyamine role as ROS's scavengers and photosynthetic apparatus protector or as defense system stimulators when plants are under water restriction (Hussain *et al.*, 2011).

At 10 DAS, the plants without any water restriction and sprayed with 50 mg L^{-1} of putrescine had significant differences in CO_2 intercellular concentrations; it could suggest that higher doses of putrescine can have a positive influence on the internal CO_2 regulation, even in plants under mild water deficit. Also, at 5 DAS, plants without water restriction and sprayed with 50 mg L^{-1} of putrescine had higher plant dry matter accumulation because of the adequate water status and higher chlorophyll contents that improve the photoassimilates directed to sink organs. In contrast, plants submitted to mild water deficit presented an improved osmotic regulation mechanism through the proline accumulation in the leaves. Furthermore, those plants had chlorophyll degradation, stomata closure and lower CO_2 assimilation, facts that possibly had an influence on dry matter accumulation. In our research, basil plants sprayed with 50 and 100 mg L^{-1} of putrescine had different responses in WUE values at 10 DAS, suggesting that duration of water stress has a pivotal role in the putrescine regulation effect and CO_2 assimilation efficiency in order to reduce the water losses in plants submitted to water deficit.

Regarding the antioxidant enzymes activity, no relevant differences were shown, neither in water levels nor putrescine doses. Medicinal plants have shown an increasing activity of the antioxidant enzymes as ROS's accumulations and it depends on the stress intensity (Mohammadi *et al.*, 2018).

Antioxidant enzymes activity was not representative in basil plants, so the

antioxidant enzymatic was not the main vegetal mechanism in order to mitigate the water stress effect. Plant cells have developed enzymatic and non/enzymatic complex mechanisms regarding the cell membrane protection against ROS's negative effects (Talbi *et al*., 2020). Non-enzymatic mechanisms such as: stomata closure and solutes accumulation could be involved in the internal protection of the plant structures submitted to mild water stress conditions.

Differential regulatory mechanisms are active in plants under drought stress, by different biological processes (Jiao *et al*., 2021), in this research the soil water availability had an influence on secondary metabolisms synthesis in basil plants, where mild water deficit had morphological and physiological changes influencing the secondary metabolites production. Under water deficit, leaf turgor decreases and there is a greater density of glands containing essential oils, resulting in a high accumulation in the amount of secondary compound (Simon *et al*., 1992).

Therefore, our results suggest that regardless of the putrescine doses, the soil water availability have a fundamental importance in the production and accumulation of secondary compounds; and the secondary compounds intensity may be linked to the stress conditions which the basil plants are exposed to.

5 CONCLUSION

Exogenous putrescine alters the morpho-physiological characteristics in basil plants submitted to adequate irrigation or regulated deficit irrigation. Putrescine, at low doses, stimulate photosynthetic pigments content, water status adjustments and higher dry matter accumulation in basil plants under mild water deficit, furthermore, it allows for positive signaling against the oxidative stress.

In contrast, soil water availability was the main factor in the essential oil profile determination. Also, the defense mechanism in basil plants submitted to a long period of mild water deficit was directly linked with the proline accumulation in the leaves.

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