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ORIGINAL ARTICLE

Water deficit modulates growth, morphology, and the essential oil profile in *Lippia alba* L. (Verbenaceae) grown in vitro

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Abstract

Lippia alba (Miller) N.E. Brown is an aromatic plant species of great economic importance due to the medicinal properties of its essential oils, which provide stress relief, respiratory and gastrointestinal disease control, and anti-inflammatory and natural sedative effects. The plant is also effective in biological control against various pathogens and in food preservation. Water deficit is the most critical abiotic factor limiting plant growth and morpho-physiological development, as well as production of secondary metabolism compounds. The objective of this work was to evaluate the effect of water deficit on growth, photosynthesis, essential oil profile, and the expression of genes related to the biosynthesis of these compounds in *L. alba* grown in vitro. Nodal segments were cultured on medium supplemented with 0, 1, 2, and 3% (w/v) polyethylene glycol for 45 days. Water stress had a negative effect on primary metabolism indicators, such as growth, leaf area, and photosynthetic rate; but a positive effect on amino acid and total protein content. Similarly, secondary metabolism exhibited an increase in linalool but a reduction in germacrene levels under water deficit. These findings provide a deeper understanding of how water deficit affects primary and secondary metabolism in *L. alba*, showing the potential of this medicinal species to adapt to soils with low water availability, while still being able to grow and synthesize essential oils.

Key message

Water deficit significantly alters the percentage of the essential oil components linalool and germacrene in *Lippia alba* plants grown in vitro.

Keywords Abiotic stress · Germacrene · Linalool · Polyethylene glycol

Introduction

Due to their sessile nature, plants are constantly subjected to various abiotic factors. Hence, recognizing these sources of stress and deploying mechanisms to counteract them is essential for their survival (Barkla et al. 2013).

Water deficit is the most critical abiotic factor limiting plant growth, their morphology, as well as physiological and biochemical processes (Bosabalidis and Kofidis 2002; Wu et al. 2008; Cairns et al. 2012; Ghotbi-Ravandi et al. 2014; Maatallah et al. 2016; Allahverdiyev 2016; Kumar et al. 2017). Growth is particularly compromised as low water availability causes loss of turgidity, thus reducing cell division and elongation (Lawlor and Cornic 2002; Jaleel et al. 2008; Ge et al. 2012). Water scarcity promotes also

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the formation of reactive oxygen species, such as superoxide radicals, singlet oxygen, hydroxyl radicals, and hydrogen peroxide, which impair protein, nucleic acid, and lipid synthesis (Mittler et al. 2004; Ozkur et al. 2009; Xie et al. 2016). Furthermore, plants respond to water deficit by inducing stomatal closure, restricting water loss by transpiration, reducing CO_2 diffusion from intercellular spaces to the chloroplast and, ultimately, decreasing photosynthetic rates (Flexas et al. 2004; Chaves et al. 2009; Li et al. 2017).

Cellular and physiological responses to water limitation allow plants to mitigate cell damage while adapting to this condition. Responses include biosynthesis and accumulation of organic solutes such as proline, which plays an important role in osmotic adjustment (Hasegawa et al. 2000; Merwad et al. 2018), and synthesis of free radical scavenging enzymes, such as peroxidase, catalase, peroxide dismutase, and ascorbate peroxidase (Sofo et al. 2015). In addition, hormonal regulation determines changes in phytohormones, such as abscisic acid, cytokinins, gibberellic acid, auxins, and ethylene (Sharp 2002; Du et al. 2010; Wilkinson and Davies 2010; Peleg et al. 2011; Xuemei et al. 2011; Wilkinson et al. 2012).

Secondary plant metabolism is also modulated by water deficit, as manifested by variations in the quality and quantity of essential oils produced by aromatic plants (Petropoulos et al. 2008; Yadav et al. 2014; Mandoulakani et al. 2017; Morshedloo et al. 2017).

The biosynthesis of essential oil components occurs via two distinct pathways: the mevalonate pathway (MEV) and the methylerythritol phosphate pathway (MEP). The MEV pathway occurs in the cytosol, where sesquiterpenes are synthesized from the precursors pyruvate and acetylcoenzyme-A. The MEP pathway occurs in the plastids, where pyruvate and glyceraldehyde-3-phosphate are used as the precursors for the synthesis of monoterpenes (Chemat et al. 2013; Pérez Zamora et al. 2018). Examples of sesqui- and monoterpene synthesis genes are, respectively, nerolidol synthase and geraniol synthase (Castro et al. 2019).

The genus *Lippia* is distributed throughout Latin America and Africa, where it has adapted to various environments. *Lippia alba* (Verbenaceae) has great economic and social importance due to its essential oil rich in bioactive compounds (Vieira et al. 2016). The oil can prevent and treat cardiovascular disease, bronchitis, cough, asthma, stomach, and intestinal disorders (Pascual et al. 2001; Lorenzi and Matos 2008; Raut and Karuppayil 2014; Amin and Hosseinzadeh 2016; Saljoughian et al. 2018); it can be used as a food preservative (Peng and Li 2014; Szczepanski and Lipski 2014; Otoni et al. 2016; Pola et al. 2016); and it can serve as a pest control product in the agrochemical industry (Pavela and Govindarajan 2017; Benelli et al. 2018). Thus, the species is of interest for various applications, from traditional medicine to modern pharmacological and agrochemical industries.

The tissue culture techniques is an important tool to access the effects of abiotic stress on plants, as it creates conditions to better isolate these effects, allowing to develop resistant or tolerant lines for abiotic stresses (Pérez-Clemente and Gómez-Cadenas 2012). Given the wide applicability of essential oils, it is important to understand how different environments alter their production and biosynthesis routes. In spite of its relevance as a medicinal species, there is limited knowledge of how abiotic factors such as water deficit modulate primary and secondary metabolism in *L. alba*. The objective of this study was to evaluate the effect of water deficit on in vitro growth, photosynthesis, essential oil profile, and the expression of genes related to the biosynthesis of these compounds in *L. alba*.

Materials and methods

Plant material

Lippia alba plants (chemotype BGEN-04) were obtained from the Germplasm Bank of the Department of Biology, Federal University of Juiz de Fora (UFJF, Juiz de Fora, MG, Brazil), they were micropropagated from original plants granted by Embrapa Genetic Resources and Biotechnology (Cenargen, Brasília, DF, Brazil). The specimens were deposited at the Leopold Krieger Herbarium (Herbarium CESJ 48,372, UFJF). The plants were propagated *in vitro* in MS medium (Murashige and Skoog 1962) supplemented with 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, and 6.5 g L^{-1} Merck agar (Merck Millipore Corp., Darmstadt, Germany). The pH of the medium was adjusted to 5.7 ± 0.01. The medium was autoclaved at 120 °C and 108 kPa for 20 min.

Effect of water deficit

Eight nodal segments (~ 2 cm in length) were inoculated into glass flasks (600 mL capacity) containing 80 mL MS medium supplemented with 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, and 6.5 g L^{-1} Merck agar. The pH of the medium was adjusted to 5.7 ± 0.01. The medium was autoclaved at 120 °C and 108 kPa for 20 min. To induce an increasingly severe water deficit, polyethylene glycol (PEG) was added to the culture medium at three concentrations: 0, 1, 2, and 3% (w/v). Cultures were maintained in a growth room at 25 ± 1 °C, at a 16-h light photoperiod (from 6 to 22 h), and constant irradiance of 41 µmol m^{-2} s^{-1} provided by two white LED bulbs (SMD 100, 18 W; Vilux®, Vitória, ES, Brazil). The flasks were sealed with rigid polypropylene lids with two vents (10 mm diameter) covered with 0.45-µm membranes (MilliSeal® AVS-045 Air Vent, Millipore Merck, Billerica, MA, USA), allowing a CO_2 exchange rate of 25 µL L^{-1} s^{-1} (Batista et al. 2017b).

After 40 days of culture (Fig. 1), the following parameters were analyzed: primary metabolites, growth, profile of essential oils, and expression of the genes *nerolidol/linalool synthase* and *geraniol synthase*. All samples were collected at 17 h.

Biometric analysis

The following biometric analysis were performed: fresh mass (g), dry mass (g) by oven-dried at 60 ºC until constant weight, total shoot length (cm), total length of the largest root (cm), and leaf area (cm^2). The latter was measured using ImageJ software (Schneider et al. 2012).

Quantification of photosynthetic pigments, carbohydrates, proteins, and amino acids

Samples from the shoots were collected, frozen in liquid nitrogen, ground, and lyophilized. To determine the concentration of photosynthetic pigments, approximately 10 mg of lyophilized tissues were used for extraction with acetone as described by Welburn (1994). For the determination of carbohydrates (sucrose, glucose, and fructose), 25 mg of lyophilized tissues were used in the extraction with methanol and evaluated as described by Fernie et al. (2001). Quantification of protein and total amino acids was conducted as suggested by Cross et al. (2006).

In vitro photosynthetic rate

Gas exchange and quantification of in vitro photosynthetic rate were performed as proposed by Costa et al. (2014) with modifications. An AQ-S151 infrared CO_2 analyzer (Qubit Systems, Kingston, ON, Canada) was used for measurements and data collection was performed using LoggerLite 1.8.1 software (Vernier Software & Technology, Beaverton, OR, USA). Reference CO_2 was calculated by inflowing air into an empty flask, pumped from the external environment at a constant air flow rate of 300 mL min^{-1}, located within an illuminated chamber (white LED bulbs). The plants were maintained in the dark for a period of 8 h prior to analysis. Soon after measuring the reference CO_2, the flasks containing the plants were coupled to the system and the CO_2 was calculated at the stabilization point. Gas exchanges were calculated by computing the difference between the reference CO_2 and the CO_2 of the plants exposed to atmospheric air. Air temperature and humidity in the flask were measured by a Spec sensor (Thermo Recorder RS-11, Takai Spec Corp., Aichi, Japan).

The in vitro photosynthetic rate (A) was calculated by the following formula:

$$A\left(\mu mol\ m^{-2}s^{-1}\right) = \frac{\Delta CO_2}{\text{Mol Flow}}$$

where, $\Delta CO_2(ppm) = \text{Reference } CO_2 - \text{Analysis } CO_2$

$$\text{Mol Flow} = \frac{\text{Air flow rate } \left(L\ min^{-1}\right)}{\left(\frac{\left(\text{Constant for perfect gases}(22.4) \times \text{Temperature (K)}\right)}{\frac{6,00,000}{\text{(Leaf dry weight per plant (g))}}}\right)}.$$

Microextraction of essential oils

For microextraction of essential oils, 300 mg of leaves were collected and stored at − 18 °C in test tubes with screw caps. After freezing, 1 mL hexane and 0.5 mL methanol were added to each sample. The samples were kept in an ultrasonic bath (Thornton-INPEC, Vinhedo, SP, Brazil) at 70 kHz and room temperature for 1 h. Subsequently, the supernatant was filtered through a sterile cotton wick. The resulting samples consisting of a 1-µL clear solution containing the extracted oils were analyzed by gas chromatography.

Qualitative analysis of essential oils

Qualitative analysis of essential oils was carried out using a gas chromatographer coupled to a mass spectrometer (GCMS-QP2010 Plus; Shimadzu, Suzhou, China) and Rtx-5MS® column (Restek, Bellefonte, PA, USA) of 30 m × 0.25 mm. The oven temperature was set to 70 °C for 3 min and then increased by 6 °C min^{-1} to 300 °C. The injector was operated in split mode (1:10) at 240 °C. The interface and mass detector were operated at 300 °C. Helium was used as the carrier gas, with a flow of 1.53 mL min^{-1}. A standard mixture of linear hydrocarbons (C_9H_{20}, $C_{10}H_{22}$…, $C_{25}H_{52}$, and $C_{26}H_{54}$) was injected under the same conditions as the samples. Identification of the constituents was performed by comparing the obtained mass spectra with those in the NIST 9.0 database (correlation > 97%) and confirmed

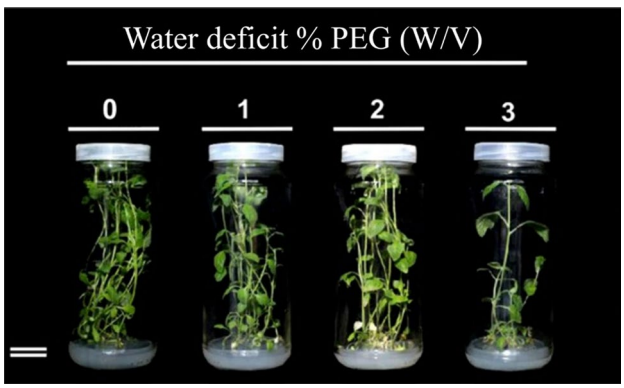
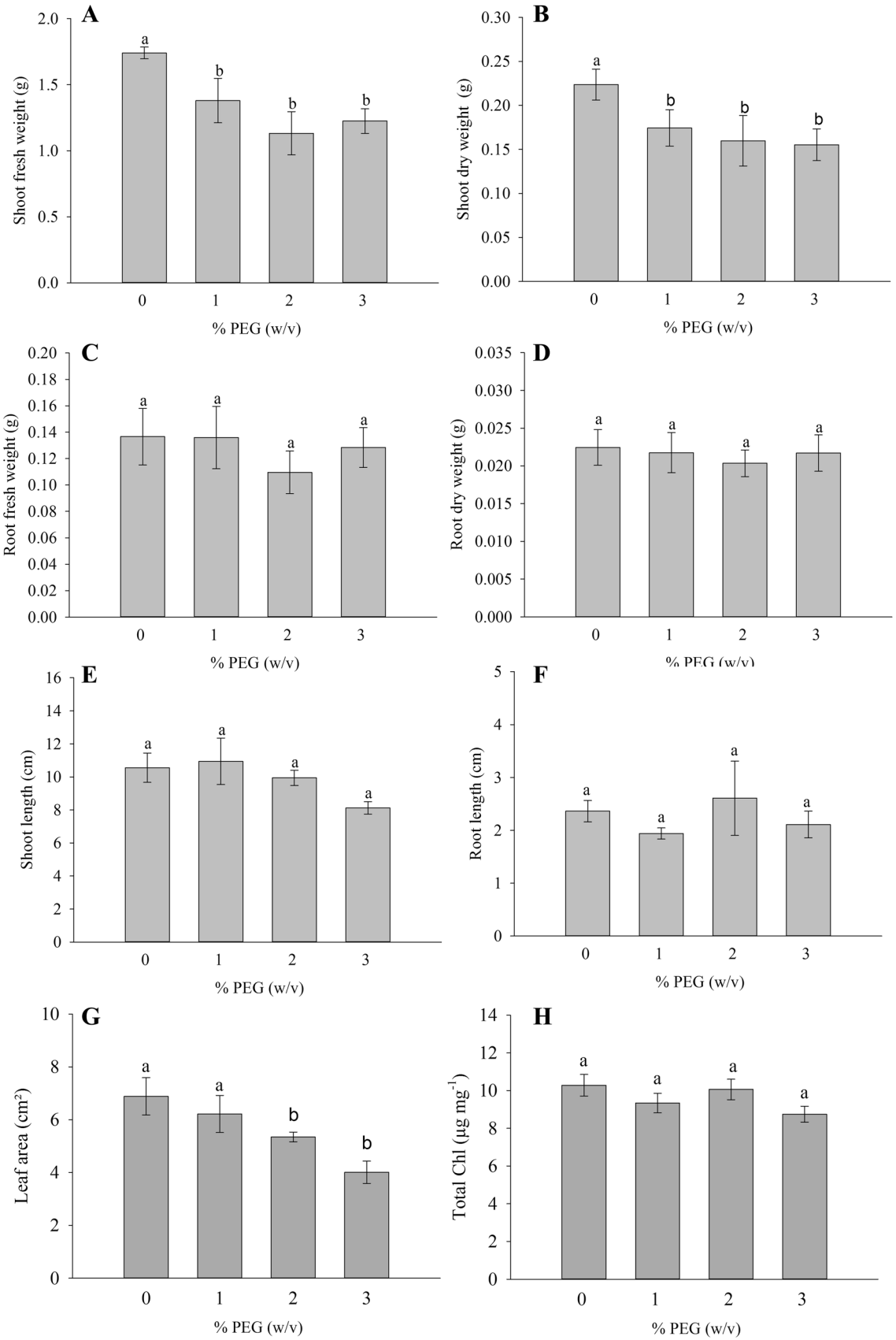


Fig. 1 Representative images of *Lippia alba* after 40 days of in vitro culture under different concentrations of PEG (0, 1, 2, and 3%). Bar = 2 cm


A
Shoot fresh weight (g)
2.0
1.5
1.0
0.5
0.0
a
b
b
b
0
1
2
3
% PEG (w/v)
B
Shoot dry weight (g)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
b
b
b
0
1
2
3
% PEG (w/v)
C
Root fresh weight (g)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
a
a
a
a
0
1
2
3
% PEG (w/v)
D
Root dry weight (g)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
a
a
a
a
0
1
2
3
% PEG (w/v)
E
Shoot length (cm)
16
14
12
10
8
6
4
2
0
a
a
a
a
0
1
2
3
% PEG (w/v)
F
Root length (cm)
5
4
3
2
1
0
a
a
a
a
0
1
2
3
% PEG (w/v)
G
Leaf area (cm²)
10
8
6
4
2
0
a
a
b
b
0
1
2
3
% PEG (w/v)
H
Total Chl (µg mg⁻¹)
14
12
10
8
6
4
2
0
a
a
a
a
0
1
2
3
% PEG (w/v)

◂**Fig. 2** Growth variables of *Lippia alba* after 40 days of in vitro culture under different concentrations of PEG (0, 1, 2, and 3%). **a** Shoot fresh weight, **b** shoot dry weight, **c** root fresh weight, **d** root dry weight, **e** shoot length, **f** root length, **g** leaf area, and **h** total chlorophyll. Data are presented as means (n = 4) and vertical bars denote the standard error. Equal letters indicate no difference according to the Scott & Knott test at 5% probability

by their retention indices (Kováts Index), which were calculated for each constituent and compared to published data (Adams 1997).

Extraction of mRNA, cDNA synthesis, and analysis by real-time PCR (RT-qPCR)

Total RNA was isolated from the leaves with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNAse I (Invitrogen) following the manufacturer's recommendations. The quality and quantity of the RNA and cDNA were determined by a NanoDrop ND-2000 (NanoDrop Technologies, Wilmington, DE, USA). cDNA was synthesized using reverse transcriptase (Ludwig Biotec, Bela Vista-Alvorada, RS, Brazil) and 800 ng of total RNA. RT-qPCR was performed on a CFX96 Touch™ cycler (Bio-Rad, Hercules, CA, USA). The total reaction volume was 10 µL:1 µL cDNA, 3 µL diethyl pyrocarbonate water, 2 µL of 4 µM forward and reverse primers, and 4 µL SYBR-Green mix/Rox (Ludwig Biotec). Primers for *nerolidol/linalool synthase (LaNES/LIS)*, *geraniol synthase (LaGES)*, and the reference gene *alcohol dehydrogenase (LaADH)* were designed based on the de novo transcriptome sequence of *L. alba*.

Statistical analysis

Experiments were conducted following a completely randomized design. The experimental unit consisted of a culture flask containing eight plants. Statistical analyses were performed using Genes software version Windows/2004.2.1 (Cruz 2016). Data were submitted to analysis of variance (ANOVA) by the F test and the means were compared by the Scott and Knott test ($P \leq 0.05$). RT-qPCR expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) with three biological replicates and at least three technical replicates. Means were compared by Dunnett's test ($P \leq 0.05$).

Results

Water deficit slows the growth of *Lippia alba* plants in vitro

Water deficit affected growth and development of *Lippia alba* grown in vitro (Fig. 1). Shoot fresh and dry mass were higher in the control than in other treatments (Fig. 2a, b); whereas root fresh/dry mass and length (Fig. 2c, d, and f), as well as stem length (Fig. 2e) exhibited no significant difference. Leaf area was higher in the control and 1% PEG condition than in the 2 and 3% PEG treatments, in with water deficit induction was more severe (Fig. 2g). Finally, total chlorophyll content (Fig. 2h) showed no significant difference.

Water deficit augments total protein and amino acid contents, but not sugar levels in *Lippia alba* grown in vitro

Glucose, fructose, and sucrose levels were not modulated by water deficit treatments (Fig. 3a–c). In contrast, total protein levels increased in the treatments with 2 and 3% PEG (Fig. 3d). Amino acid levels were higher in plants exposed to 2% PEG (Fig. 3e).

Severe water deficit reduces *Lippia alba* photosynthesis in vitro

In vitro photosynthesis of *L. alba* was reduced in plants grown in the presence of 3% PEG, and manifested as a significant decrease in photosynthetic rates compared to the other treatments (Fig. 4).

Linalool content increases in *Lippia alba* plants grown in vitro under water deficit

Water deficit changed the profile of essential oils in *L. alba*. Specifically, linalool content was significantly higher in plants subjected to water deficit induced by different levels of PEG than in control plants (Fig. 5); whereas germacrene content exhibited the opposite trend and was significantly higher in the control. Eucalyptol content did not differ statistically among the treatments.

Water deficit does not alter the expression of nerolidol/linalool synthase and geraniol synthase in *Lippia alba*

The relative expression of *LaNES/LIS* and *LaGES* was not altered by different water deficit levels (Fig. 6).

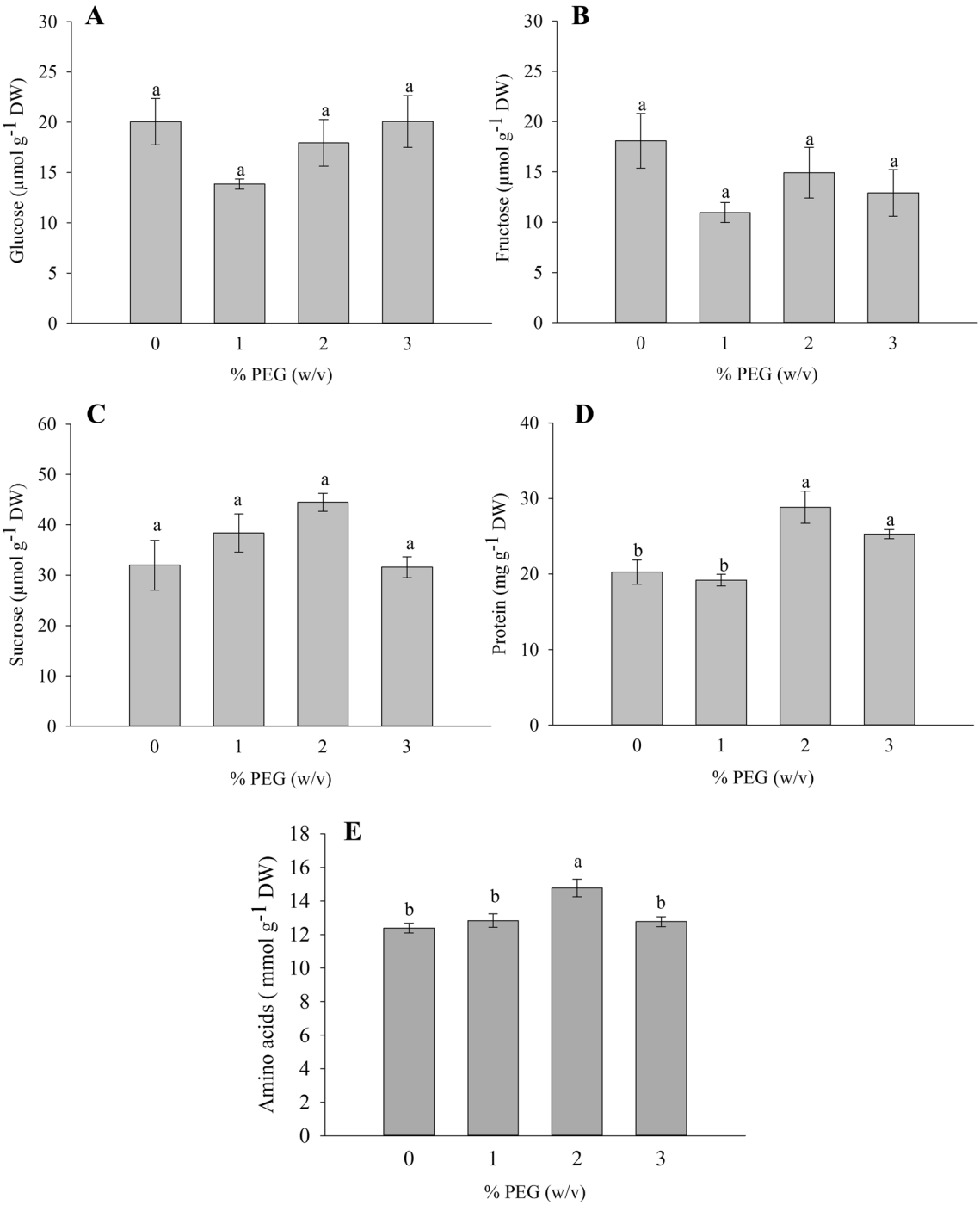


Fig. 3 Sugar, protein, and amino acid contents of *Lippia alba* after 40 days of in vitro culture under different concentrations of PEG (0, 1, 2, and 3%). **a** Glucose, **b** fructose, **c** sucrose, **d** protein, and **e** amino acids. Data are presented as means (n = 4) and vertical bars denote the standard error. Equal letters indicate no difference according to the Scott and Knott test at 5% probability

Discussion

Abiotic factors are constantly modulating the primary and secondary metabolism of plants, affecting yield and/or composition of essential oils (Bahreininejad et al. 2014; Batista et al. 2016, 2017a; Mahmoud et al. 2018). Several studies have found a correlation between water deficit and essential oils profile (Yadav et al. 2014; Mandoulakani et al. 2017; Morshedloo et al. 2017). However, the present study is the first to provide a comprehensive picture of the effect of water deficit on primary metabolism, growth, photosynthesis, the

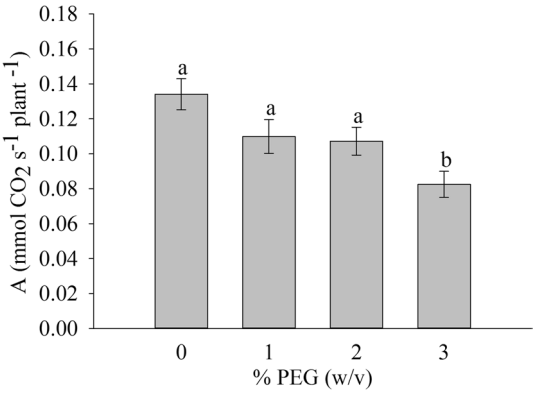


Fig. 4 Photosynthetic rate (a) of *Lippia alba* after 40 days of in vitro culture under different concentrations of PEG (0, 1, 2, and 3%). Data are presented as means (n = 4) and vertical bars denote the standard error. Equal letters indicate no difference according to the Scott and Knott test at 5% probability

profile of essential oils, and the expression of genes related to their biosynthesis in *L. alba* grown in vitro.

Regarding primary metabolism, this work evidences how the more severe water deficit treatments, using 2 and 3% PEG, promoted a decrease in shoot fresh/dry mass and reduced the leaf area. These results confirmed earlier observations by Ghotbi-Ravandi et al. (2014) on the negative effect of water deficit on the dry matter of *Hordeum vulgare* L. genotypes, and by Maatallah et al. (2016) on the decrease in relative growth rate, leaf area, and chlorophyll content following water deficit in *Laurus nobilis* L. Similarly, Allahverdiyev (2016) reported a reduction of leaf area in wheat cultivars subjected to water deficit. In general, the reduction in plant biomass caused by water deficit is related to a suppression of cell expansion. Specifically, the decrease in turgor pressure caused by lower cell water content results in reduced growth (Jaleel et al. 2008). Moreover, reducing leaf expansion is one of the strategies employed by plants to mitigate low water availability, resulting in decreased leaf area, stomatal closure, lower transpiration, and limited photosynthesis (Ge et al. 2012).

In *L. alba* plants grown in vitro, the water deficit elicited by treatment with 3% PEG promoted a reduction of photosynthetic rate. This response could be caused by limited CO_2 diffusion from intercellular spaces to the chloroplast, which may constrain CO_2 fixation (Flexas et al. 2004; Chaves et al. 2009). Water restriction often culminates in stomatal closure, an effective mechanism against water loss (Cornic 2000; Loreto et al. 2003; Molnar et al. 2005; Chaves et al. 2009), this effect was already observed in *Lippia alba* plants in vivo under water stress, where there was a decrease in stomatal conductance (Oliveira and Leite 2017) Li et al. (2017). observed a decreased photosynthetic rate in potato seedlings under severe water deficit, suggesting that this reduction was due to stomatal limitation and the damage to photosystem II and antioxidant enzymes. Ghotbi-Ravandi et al. (2014) reported a reduction in both CO_2 assimilation rate and stomatal conductance in barley genotypes under severe water stress.

Here, water deficit did not affect the levels of glucose, fructose, and sucrose, but the 2% PEG treatment increased total amino acids content. In dry conditions, many metabolites accumulate to act as regulatory osmolytes. This is one of the mechanisms that plants develop to counteract environmental stresses such as drought (Seki et al. 2007). Sugars (raffinose family oligosaccharides, sucrose, trehalose, and sorbitol), amino acids (proline), sugar alcohols (mannitol),

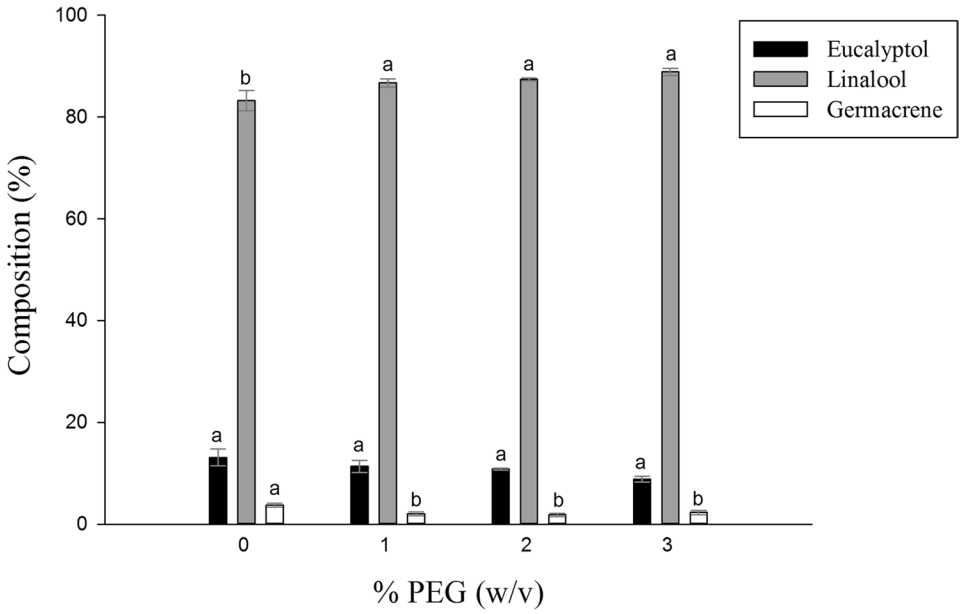


Fig. 5 Profile of essential oils in *Lippia alba* after 40 days of in vitro culture under different concentrations of PEG (0, 1, 2, and 3%). Data are presented as means (n = 4) and vertical bars denote the standard error. Equal letters indicate no difference according to the Scott and Knott test at 5% probability

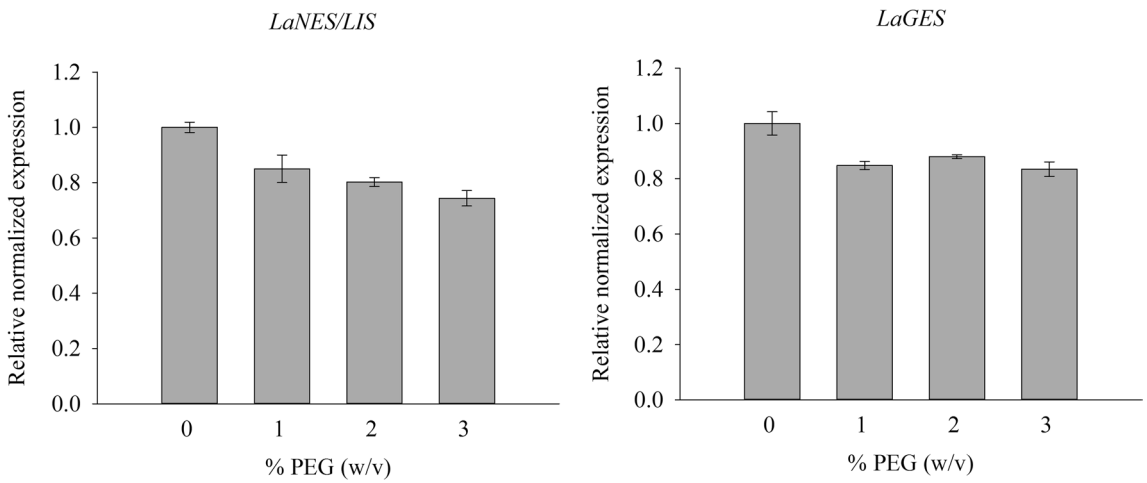


Fig. 6 *Geraniol synthase (LaGES)* and *neralidol/linalool synthase (LaNES/LIS)* expression levels in *Lippia alba* after 40 days of in vitro culture under different concentrations of PEG (0, 1, 2, and 3%). Expression is normalized to that of *alcohol dehydrogenase (LaADH)*. Data are presented as means (n = 3) and are compared by Dunnett's test at 5% probability, vertical bars denote the standard error

and amines (glycine, betaine, and polyamines) are considered compatible solutes that can be concentrated in the cell cytoplasm to promote water balance between vacuole and cytoplasm (Per et al. 2017; Merwad et al. 2018; Naeem et al. 2018).

Total protein content increased in plants subjected to moderate and severe water deficit conditions. Xie et al. (2016) observed that, compared to controls, water deficit increased and decreased the content of 260 and 206 proteins, respectively. Most of these proteins were involved in photosynthesis, metabolism, stress, and defense, indicating that water stress could modulate protein synthesis and influence plant physiology, promoting different stress responses.

Treatments in which water deficit was induced by 1, 2, and 3% PEG influenced the profile of essential oils, increasing linalool content but decreasing germacrene content. Similar effects under severe water stress have also been reported earlier, with increased content of methyl chavicol, methyl eugenol, β-myrcene, and α-bergamotene in *Ocimum basilicum* L. (Mandoulakani et al. 2017). Morshedloo et al. (2017) also reported that water deficit up- and down-regulated specific essential oil components in subspecies of *Origanum vulgare* L. resulting in increased content of essential oil. Yadav et al. (2014) observed a reduction in the density of glandular trichomes in leaves of *Artemisia annua* L., with a consequent decrease in the content of essential oils. On the other hand, Cruz et al. (2014), in a study with *Lippia gracilis* plants in the field, found no variation among plants subjected to different irrigation conditions, which highlights the importance of in vitro studies that offer more controlled conditions to detect subtle variations in secondary metabolism.

The relative expression of genes involved in the biosynthesis of *L. alba* essential oil components was not significant in the present study. This finding contrasts with that by Mandoulakani et al. (2017), who observed that essential oil biosynthesis genes in *Ocimum basilicum* L. were differentially expressed under the most severe water stress condition (50% field capacity). Specifically, *O-methyl transferase* and *eugenol O-methyl transferase* expression were increased by approximately 6.0 and 46.0 fold, respectively, which correlated strongly with the content of the essential oil compounds methyl chavicol and methyleugenol. At the same time, this increase was accompanied by a reduction in the expression of *4-coumarate:CoA ligase* and *cinnamate 4-hydroxylase*, two enzymes involved in the metabolism of hydroxycinnamic acid.

In conclusion, our results show that water deficit is capable of modulating the primary metabolism of *Lippia alba*, as indicated by a reduction in growth, development, and physiological functions. Secondary metabolism was also slightly altered, with linalool and germacrene levels modified in plants under water deficit. Thus, the medicinal species *L. alba* can adapt to low water availability environments, while still being able to grow and produce essential oils.

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Author contributions KMC and DSB conceived and designed the experiments; KMC raised the in vitro plants; TDS, EAF, SHSF, AMF, and RMJS performed the morphoanatomical and physiological analyses; KMC, LSQN, VRC, and RMG performed the chemical analyses;

KMC, DSB, LFV, RMG, and WCO contributed to the design and interpretation of the research and to the writing of the paper. All authors have read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Adams RP (1997) Identification of essential oil components by gas chromatography/mass spectroscopy. J Am Soc Mass Spectrom 6:671–672

Allahverdiyev TI (2016) Impact of soil water deficit on some physiological parameters of durum and bread wheat genotypes. Agric For 62:131–144. https://doi.org/10.17707/AgricultForest.62.1.16

Amin B, Hosseinzadeh H (2016) Black cumin (*Nigella sativa*) and its active constituent, thymoquinone: an overview on the analgesic and anti-inflammatory effects. Planta Med 82:8–16. https://doi.org/10.1055/s-0035-1557838

Bahreininejad B, Razmjoo J, Mirza M (2014) Effect of water stress on productivity and essential oil content and composition of *Thymus carmanicus*. J Essent Oil-Bear Plants 17:717–725. https://doi.org/10.1080/0972060X.2014.901605

Barkla BJ, Vera-Estrella R, Pantoja O (2013) Progress and challenges for abiotic stress proteomics of crop plants. Proteomics 13:1801–1815. https://doi.org/10.1002/pmic.201200401

Batista DS, Castro KM, Silva AR, Teixeira ML, Sales TA, Soares LI, Cardoso MG, Santos MO, Viccini LF, Otoni WC (2016) Light quality affects in vitro growth and essential oil profile in Lippia alba (Verbenaceae). In Vitro Cell Dev Biol-Plant 52:276–282. https://doi.org/10.1007/s11627-016-9761-x

Batista DS, Castro KM, Koehler AD, Porto BN, Silva AR, Souza VC, Teixeira ML, Cardoso MG, Santos MO, Viccini LF, Otoni WC (2017a) Elevated CO_2 improves growth, modifies anatomy, and modulates essential oil qualitative production and gene expression in *Lippia alba* (Verbenaceae). Plant Cell Tissue Organ Cult 128:357–368. https://doi.org/10.1007/s11240-016-1115-1

Batista DS, Dias LLC, Rêgo MMD, Saldanha CW, Otoni WC (2017b) Flask sealing on in vitro seed germination and morphogenesis of two types of ornamental pepper explants. Cienc Rural 47:1–6. https://doi.org/10.1590/0103-8478cr20150245

Benelli G, Pavela R, Giordani C, Casettari L, Curzi G, Cappellacci L, Petrelli R, Maggi F (2018) Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. Ind Crops Prod 112:668–680. https://doi.org/10.1016/j.indcrop.2017.12.062

Bosabalidis AM, Kofidis G (2002) Comparative effects of drought stress on leaf anatomy of two olive cultivars. Plant Sci 163:375–379. https://doi.org/10.1016/S0168-9452(02)00135-8

Cairns JE, Sanchez C, Vargas M, Ordoñez R, Araus JL (2012) Dissecting maize productivity: ideotypes associated with grain yield under drought stress and well-watered conditions. J Integr Plant Biol 54:1007–1020. https://doi.org/10.1111/j.1744-7909.2012.01156.x

Castro KM, Batista DS, Fortini EA, Silva TD, Felipe SHS, Fernandes AM, Sousa RMJ, Nascimento LSQ, Campos VR, Grazul RM, Viccini LF, Otoni WC (2019) Photoperiod modulates growth, morphoanatomy, and linalool content in *Lippia alba* L. (Verbenaceae) cultured in vitro. Plant Cell Tissue Organ Cult 139:139–153. https://doi.org/10.1007/s11240-019-01672-w

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560. https://doi.org/10.1093/aob/mcn125

Chemat F, Maryline A-V, Xavier F (2013) Microwave-assisted extraction of essential oils and aromas. Microwave-assisted extraction for bioactive compounds. Springer, New York, pp 53–68. https://doi.org/10.1007/978-1-4614-4830-3_3

Cornic G (2000) Drought stress inhibits photosynthesis by decreased stomatal aperture not by affecting ATP synthesis. Trends Plant Sci 5:187–188. https://doi.org/10.1016/S1360-1385(00)01625-3

Costa AC, Rosa M, Megguer CA, Silva FG, Pereira FD, Otoni WC (2014) A reliable methodology for assessing the *in vitro* photosynthetic competence of two Brazilian savanna species: *Hyptis marrubioides* and *Hancornia speciosa*. Plant Cell Tissue Organ Cult 117:443–454. https://doi.org/10.1007/s11240-014-0455-y

Cross JM, Von Korff M, Altmann T, Bartzetko L, Sulpice R, Gibon Y, Palacios N, Stitt M (2006) Variation of enzyme activities and metabolite levels in 24 *Arabidopsis* accessions growing in carbon-limited conditions. Plant Physiol 142:1574–1588. https://doi.org/10.1104/pp.106.086629

Cruz CD (2016) Genes software-extended and integrated with the R, Matlab and Selegen. Acta Sci Agron 38:547–552. https://doi.org/10.4025/actasciagron.v38i4.32629

Cruz EMDO, Pinto JAO, Fontes SS, Arrigoni-Blank MDF, Bacci L, Jesus HCRD, Santos DA, Alves PB, Blank AF (2014) Water deficit and seasonality study on essential oil constituents of *Lippia gracilis* Schauer germplasm. TSWJ 2014:1–9. https://doi.org/10.1155/2014/314626

Du H, Wang N, Cui F, Li X, Xiao J, Xiong L (2010) Characterization of a β-carotene hydroxylase gene *DSM2* conferring drought and oxidative stress resistance by increasing xanthophylls and ABA synthesis in rice. Plant Physiol 154:1304–1318. https://doi.org/10.1104/pp.110.163741

Fernie AR, Roscher A, Ratcliffe RG, Kruger NJ (2001) Fructose 2,6-bisphosphate activates pyrophosphate: fructose-6-phosphate 1-phosphotransferase and increases triose phosphate to hexose phosphate cycling in heterotrophic cells. Planta 212:250–263. https://doi.org/10.1007/s004250000386

Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD (2004) Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol 6:269–279. https://doi.org/10.1055/s-2004-820867

Ge T, Sui F, Bai L, Tong C, Sun N (2012) Effects of water stress on growth, biomass partitioning, and water-use efficiency in summer maize (*Zea mays* L.) throughout the growth cycle. Acta Physiol Plant 34:1043–1053. https://doi.org/10.1007/s11738-011-0901-y

Ghotbi-Ravandi AA, Shahbazi M, Shariati M, Mulo P (2014) Effects of mild and severe drought stress on photosynthetic efficiency in tolerant and susceptible barley (*Hordeum vulgare* L.) genotypes. J Agron Crop Sci 200:403–415. https://doi.org/10.1111/jac.12062

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Ann Rev Plant Biol 51:463–499. https://doi.org/10.1146/annurev.arplant.51.1.463

Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R (2008) Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. Colloid Surf B 61:298–303. https://doi.org/10.1016/j.colsurfb.2007.09.008

Kumar A, Nayak AK, Pani DR, Das BS (2017) Physiological and morphological responses of four different rice cultivars to soil water potential based deficit irrigation management strategies. Field Crops Res 205:78–94. https://doi.org/10.1016/j.fcr.2017.01.026

Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher

plants. Plant Cell Environ 25:275–294. https://doi.org/10.1046/j.0016-8025.2001.00814.x
Li J, Cang Z, Jiao F, Bai X, Zhang D, Zhai R (2017) Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. J Saudi Soc Agric Sci 16:82–88. https://doi.org/10.1016/j.jssas.2015.03.001
Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
Lorenzi H, Matos FJA (2008) Plantas Medicinais no Brasil—Nativas e Exóticas. Instituto Plantarum de Estudos da Flora, Nova Odessa, p 512
Loreto F, Centritto M, Chartzoulakis K (2003) Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. Plant Cell Environ 26:595–601. https://doi.org/10.1046/j.1365-3040.2003.00994.x
Maatallah S, Nasri N, Hajlaoui H, Albouchi A, Elaissi A (2016) Evaluation changing of essential oil of laurel (*Laurus nobilis* L.) under water deficit stress conditions. Ind Crops Prod 91:170–178. https://doi.org/10.1016/j.indcrop.2016.07.001
Mahmoud AA, Gendy ASH, Said-Al Ahl HAH, Grulova D, Astatkie T, Abdelrazik TM (2018) Impacts of harvest time and water stress on the growth and essential oil components of horehound (*Marrubium vulgare*). Sci Hortic 232:139–144. https://doi.org/10.1016/j.scienta.2018.01.004
Mandoulakani BA, Eyvazpour E, Ghadimzadeh M (2017) The effect of drought stress on the expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil components in basil (*Ocimum basilicum* L.). Phytochemistry 139:1–7. https://doi.org/10.1016/j.phytochem.2017.03.006
Merwad ARM, Desoky ESM, Rady MM (2018) Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application. Sci Hortic 228:132–144. https://doi.org/10.1016/j.scienta.2017.10.008
Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498. https://doi.org/10.1016/j.tplants.2004.08.009
Molnar I, Dulai S, Csernak A, Pronay J, Molnar-Lang M (2005) Photosynthetic responces to drought stress in different *Aegilops* species. Acta Biol Szeged 49:141–142
Morshedloo MR, Craker LE, Salami A, Nazeri V, Sang H, Maggi F (2017) Effect of prolonged water stress on essential oil content, compositions and gene expression patterns of mono-and sesquiterpene synthesis in two oregano (*Origanum vulgare* L.) subspecies. Plant Physiol Biochem 111:119–128. https://doi.org/10.1016/j.plaphy.2016.11.023
Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
Naeem M, Naeem MS, Ahmad R, Ihsan MZ, Ashraf MY, Hussain Y, Fahad S (2018) Foliar calcium spray confers drought stress tolerance in maize via modulation of plant growth, water relations, proline content and hydrogen peroxide activity. Arch Agron Soil Sci 64:116–131. https://doi.org/10.1080/03650340.2017.1327713
Oliveira PC, Leite MN (2017) Amazonian medicinal herbs: physiology of stress. J Med Plants 5:255–264
Otoni CG, Espitia PJ, Avena-Bustillos RJ, McHugh TH (2016) Trends in antimicrobial food packaging systems: Emitting sachets and absorbent pads. Food Res Int 83:60–73. https://doi.org/10.1016/j.foodres.2016.02.018
Ozkur O, Ozdemir F, Bor M, Turkan I (2009) Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovate* Desf. to drought. Environ Exp Bot 66:487–492. https://doi.org/10.1016/j.envexpbot.2009.04.003
Pascual ME, Slowing K, Carretero E, Sánches Mata D, Villar A (2001) *Lippia*: traditional uses, chemistry and pharmacology: a review. J Ethnopharm 76:201–214. https://doi.org/10.1016/S0378-8741(01)00234-3
Pavela R, Govindarajan M (2017) The essential oil from *Zanthoxylum monophyllum* a potential mosquito larvicide with low toxicity to the non-target fish *Gambusia affinis*. J Pest Sci 90:369–378. https://doi.org/10.1007/s10340-016-0763-6
Peleg Z, Reguera M, Tumimbang E, Walia H, Blumwald E (2011) Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. Plant Biotechnol J 9:747–758. https://doi.org/10.1111/j.1467-7652.2010.00584.x
Peng Y, Li Y (2014) Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. Food Hydrocoll 36:287–293. https://doi.org/10.1016/j.foodhyd.2013.10.013
Per TS, Khan NA, Reddy PS, Masood A, Hasanuzzaman M, Khan MIR, Anjum NA (2017) Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: phytohormones, mineral nutrients and transgenics. Plant Physiol Biochem 115:126–140. https://doi.org/10.1016/j.plaphy.2017.03.018
Pérez-Clemente RM, Gómez-Cadenas A (2012) In vitro tissue culture, a tool for the study and breeding of plants subjected to abiotic stress conditions. In: Recent advances in plant in vitro culture. IntechOpen. https://doi.org/10.5772/50671
Pérez Zamora CM, Torres CA, Nuñez MB (2018) Antimicrobial activity and chemical composition of essential oils from Verbenaceae species growing in South America. Molecules 23:544. https://doi.org/10.3390/molecules23030544
Petropoulos SA, Daferera D, Polissiou MG, Passam HC (2008) The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. Sci Hortic 115:393–397. https://doi.org/10.1016/j.scienta.2007.10.008
Pola CC, Medeiros EA, Pereira OL, Souza VG, Otoni CG, Camilloto GP, Soares NF (2016) Cellulose acetate active films incorporated with oregano (*Origanum vulgare*) essential oil and organophilic montmorillonite clay control the growth of phytopathogenic fungi. Food Pack Shelf Life 9:69–78. https://doi.org/10.1016/j.fpsl.2016.07.001
Raut JS, Karuppayil SM (2014) A status review on the medicinal properties of essential oils. Ind Crops Prod 62:250–264. https://doi.org/10.1016/j.indcrop.2014.05.055
Saljoughian S, Shahin R, Alaa El-Din AB, Ralf G, Alireza O, Nooshin N, Amin MK (2018) The effects of food essential oils on cardiovascular diseases: a review. Crit Rev Food Sci Nutr 58:1688–1705. https://doi.org/10.1080/10408398.2017.1279121
Seki M, Umezawa T, Urano K, Shinozaki K (2007) Regulatory metabolic networks in drought stress responses. Curr Opin Plant Biol 10:296–302. https://doi.org/10.1016/j.pbi.2007.04.014
Sharp RE (2002) Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. Plant Cell Environ 25:211–222. https://doi.org/10.1046/j.1365-3040.2002.00798.x
Sofo A, Scopa A, Nuzzaci M, Vitti A (2015) Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. Int J Mol Sci 16:13561–13578. https://doi.org/10.3390/ijms160613561
Szczepanski S, Lipski A (2014) Essential oils show specific inhibiting effects on bacterial biofilm formation. Food Control 36:224–229. https://doi.org/10.1016/j.foodcont.2013.08.023
Vieira RF, Silva DB, Salimena FRG (2016) Lippia alba—Erva-cidreira. In: Vieira RF, Camillo J, Coradin L (eds) Espécies nativas da flora brasileira de valor econômico atual ou potencial—Plantas para o futuro: Região Centro-Oeste. MMA, Brasília, pp 383–394
Welburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with

spectrophotometers of different resolution. J Plant Physiol 144:307–313. https://doi.org/10.1016/S0176-1617(11)81192-2

Wilkinson S, Davies WJ (2010) Drought, ozone, ABA and ethylene: new insights from cell to plant to community. Plant Cell Environ 33:510–525. https://doi.org/10.1111/j.1365-3040.2009.02052.x

Wilkinson S, Kudoyarova GR, Veselov DS, Arkhipova TN, Davies WJ (2012) Plant hormone interactions: innovative targets for crop breeding and management. J Exp Bot 63:3499–3509. https://doi.org/10.1093/jxb/ers148

Wu F, Bao W, Li F, Wu N (2008) Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. Environ Exp Bot 63:248–255. https://doi.org/10.1016/j.envexpbot.2007.11.002

Xie H, Yang DH, Yao H, Bai GE, Zhang YH, Xiao BG (2016) iTRAQ-based quantitative proteomic analysis reveals proteomic changes in leaves of cultivated tobacco (*Nicotiana tabacum*) in response to drought stress. Biochem Biophys Res Commun 469:768–775. https://doi.org/10.1016/j.bbrc.2015.11.133

Xuemei J, Dong B, Shiran B, Talbot MJ, Edlington JE, Trijntje H, Rosemary GW, Frank G, Dolferus R (2011) Control of ABA catabolism and ABA homeostasis is important for reproductive stage stress tolerance in cereals. Plant Physiol 156:647–662. https://doi.org/10.1104/pp.111.176164

Yadav RK, Sangwan RS, Sabir F, Srivastava AK, Sangwan NS (2014) Effect of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in *Artemisia annua* L. Plant Physiol Biochem 74:70–83. https://doi.org/10.1016/j.plaphy.2013.10.023