Scientia Horticulturae 319 (2023) 112187

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Physiological and biochemical changes in response to *Moringa oleifera* biostimulant in petunia plants under water deficit

S. Toscano [a], M.J. Gómez-Bellot [b], D. Romano [c,*], M.J. Sánchez-Blanco [b]

[a] Department of Veterinary Sciences, Università degli Studi di Messina, Polo Universitario Annunziata, Messina 98168, Italy
[b] Department of Irrigation, Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), P.O. Box 164, Espinardo, Murcia 30100, Spain
[c] Department of Agriculture, Food and Environment (Di3A), Università degli Studi di Catania, Via Valdisavoia 5, Catania 95123, Italy

ARTICLE INFO

Keywords:
Petunia hybrida E.Vilm.
Gas exchange
Water use efficiency
Biostimulants
Antioxidant activity
Phytohormones

ABSTRACT

The present paper aimed to evaluate the effects of LME (*Moringa oleifera* leaf extract) in modifying growth, ornamental value and several physiological and chemical parameters of petunia (*Petunia hybrida* E.Vilm.) 'GO! Tunia® Neon Pink'. Three level of water deficit were considered: control (100% of WCC, Water Container Capacity = 100% CC), 60% and 40% of WCC; for each level treatments with LME were also considered. Water deficit reduced growth parameters compared with full irrigation and LME application promoted almost all the growth parameters in both control and stressed conditions. The behavior in growth parameters is correlated with a decrease in photosynthesis activity and plant water status. Deficit irrigation reduced the Relative Water Content (RWC) without differences linked to LME application. The chlorophyll content was unchanged for effect of water deficit and enhanced by LME treatment. The capacity to accumulate protective compounds (Proline, MDA) allowed plant to reduce the negative effects of water stress; LME treatment is not always able to increase these compounds in more stressed plants. LME application increased GPX and SOD activities in plants grown under drought stress, and this facilitated the ROS scavenging and maintenance of plant growth under stress. Total phenol compounds (TPC) showed significant differences in relation to the water deficit treatments, but not to biostimulants; the interaction effect was significant. Total soluble sugars in the leaf tissues were significantly affected only by deficit irrigation treatments. Drought stress also affected the production of endogenous level of hormones and amino acid. The highest content for almost all free amino acids was observed in the most stressed treatment (40% CC and 40% CC + LME). The response of petunia plants to water deficit was related to its ability to decrease aerial growth and to modify leaf gas exchange, increasing secondary osmolytes and enzyme activity to contrast the ROS activity.

1. Introduction

Global climate change and the associated unfavorable abiotic stress conditions, such as drought, salinity, heavy metals, and extreme temperatures, greatly affect plant growth and development. In the Mediterranean regions, water deficit is one of the main problems for ornamental plant use, and global changes will predictably amplify the present issues, especially in urban areas (Fahad et al., 2017; WWAP, 2014).

The application of deficit irrigation strategies in floriculture can make a significant contribution to the conservation of irrigation water (Sánchez-Blanco et al., 2019). In the near future, global warming will increase the frequency and severity of drought (Lin and Raza, 2019). Therefore, in a changing climate, the study of the main physiological limits to productivity in drought conditions will be crucial to improve yield stability. Since the increased frequency of drought negatively affects plant growth and development (Lobell and Gourdji, 2012), analyzing the effects of water deficit on plants is important to hypothesize the influence that future climate change will have on growth of a particular plant species (Farooq et al., 2009). It is important to understand the physiological and biochemical responses of different plant species to moderate and severe water stress in order to identify the

Abbreviations: CC, Container capacity; SLA, Specific leaf area; A_N, Net CO_2 assimilation rate; Fv/Fm, Maximum quantum efficiency of PSII; g_s, Stomatal conductance; WUE, Water use efficiency; RWC, Relative water content; PRO, Proline; LME, *Moringa oleifera* leaf extract; MDA, Malondialdehyde; CAT, Catalase; GPX, Glutathione peroxidase; SOD, Superoxide dismutase; TPC, Total phenol compounds; ROS, Reactive Oxigen Species; E, Transpiration rate.

* Corresponding author.
E-mail address: dromano@unict.it (D. Romano).

https://doi.org/10.1016/j.scienta.2023.112187
Received 23 January 2023; Received in revised form 3 May 2023; Accepted 25 May 2023

threshold of stress levels (Yadollahi et al., 2011) and the controlled application of the water may be used in potted plants also to improve quality (Cameron et al., 2006).

Over the last decades, many studies have reported morphological, physiological and biochemical changes in plant responses to water deficit (Tribulato et al., 2019; Chaves et al., 2003). In particular, plant response may involve metabolic pathways such as photosynthesis, sugar synthesis, tricarboxylic acid cycle, glycolysis and hormone synthesis (Guo et al., 2018). The reduced moisture availability induces negative changes in photosynthetic pigments, damages the photosynthetic machinery (Fu and Huang, 2001), and the thylakoid membranes (Anjum et al., 2011). The reduction of chlorophyll contents under drought conditions has also been reported (Din et al., 2011). Exposure of plants to drought stresses initially causes oxidative damage by the formation of ROS. In order to cope with the oxidative stress, plants usually rely on the antioxidant defense, which can be either enzymatic or non-enzymatic. Enzymatic defense is usually considered as the most effective (Farooq et al., 2009). Major enzymes involved in this system are SOD, GR, POD, and CAT (Farooq et al., 2009). Beside these enzymes, certain carotenoids and glutathione can also play part in the antioxidant system as non-enzymatic components.

Among hormone production in stress abiotic conditions, abscisic acid (ABA) is the primary chemical signal for drought, increasing in concentration and inducing stomatal closure to minimize water loss. ABA also alters the expression of a multitude of drought stress-related genes (Bray, 2004). Furthermore, plants subjected to water deficit may reduce flowering production, bring forward, or delay flowering and shorten the same (Álvarez et al., 2013). This should be borne in mind in the case of ornamental plants because the most decorative elements in this kind of plant are usually flowers.

However, the severity of drought stress is also a critical factor that determines plant response; plant responses to drought stress that are generally evident under mild and/or moderate water stress may be absent under severe stress (Watkinson et al., 2003). In petunia plants, physiological and molecular responses demonstrated differences in plants in relation to varying severity of drought stress. Plants at $\theta < 0.40$ $m3{\cdot}m^{-3}$ displayed an increase in leaf ABA concentrations; however, no significant changes in the relative expression of ABA biosynthesis-related genes were observed in plants under severe drought stress (Kim et al., 2012). In geranium moderate deficit irrigation reduced the consumption of water, while maintaining the good overall quality of plants. However, when SDI was applied, a reduction in the number of flowers per plant was observed (Sánchez-Blanco et al., 2009).

To counteract abiotic stress, in recent years, the use of biostimulant products represents a valid technological innovation and has been proposed as an agronomic tool with great potential for the sustainable development of plant production (Bulgari et al., 2019). Natural biostimulants as eco-friendly materials include any elements applied to plants with the aim of enhancing nutritional efficiency, abiotic stress tolerance and/or crop quality traits (Trivedi et al., 2018). These substances are arousing great interest in sustainable agriculture because their applications activate various physiological processes that improve the efficiency in the use of nutrients, stimulate plant growth, and allow the reduction of fertilizer consumption (Kunicki et al., 2010; Bulgari et al., 2015).

In this context, *Moringa oleifera* Lam. has been identified as a potential font of bioactive compounds for the preparation of biostimulants. Leaf extracts of *M. oleifera* applied to seeds and/or as a foliar spray, both under normal and stressful conditions, can positively modify growth and production by modifying metabolic processes (Howladar et al., 2014; Rady et al., 2013). Most biostimulants increase the content of photosynthetic pigments (chlorophyll and carotenoids) and decrease the content of polyphenols and antioxidant radicals (Godlewska et al., 2019). Biostimulants can promote the growth of ornamental plants (e.g., *Zinnia elegans* and *Petunia hybrida*) (Bayona-Morcillo et al., 2020) during production (Saini et al., 2019) and improve yield performance under abiotic stress (Lin and Jones, 2022). Moringa leaf extract has been used extensively to reduce the effects of biotic and abiotic stress (Abd El-Mageed et al., 2017). Despite being a species of tropical origin, specimens of the species are present as ornamental plants in Sicily, so it is possible to have fresh leaves (Romano et al., 2022).

Among the summer flowering bedding plants, petunia (*Petunia hybrida* E.Vilm.) is one of the most popular, a species widely appreciated for its long profusion of brightly colored flowers and good adaptability to the conditions of the Mediterranean summer. It belongs to the Solanaceae family and it is native to South America and in particular to Brazil, Argentina, Uruguay, Paraguay and Bolivia (Kulcheski et al., 2006).

For these reasons, this study hypothesized that LME application may enhance the petunia growth, biomass, and blooming, directly linked to ornamental value of the plants. In addition, the potential effects of LME on several physiological and chemical parameters, i.e., photosynthetic pigments, total phenol contents, antioxidant activity were also evaluated to understand the action mechanisms adopted by plants to reduce the effects of different water deficit levels.

2. Materials and methods

2.1. Experimental design

The trial was established in a cold greenhouse in Catania (southern Italy, 37°31N 15°04E; 20 m a.s.l.) on *Petunia hybrida*. E.Vilm. Petunia 'GO!Tunia® Neon Pink' rooted cuttings were transplanted into Ø 14 cm pots (one plant per pot) at the age of two true leaf, filled with peat and perlite substrate (2/1, v/v), and fertilized with 2 g L^{-1} of Osmocote Plus (14/13/13, N, P, K + microelements).

Plants were grouped into three repetitions of six plants per treatment (108 plants in total) and irrigated every two days. Six treatments were considered: control (100% CC), in which the substrate moisture was maintained close to container capacity and irrigated at 100% of WCC (Water Container Capacity); Control +LME (100% CC+LME), irrigated at 100% of WCC plus treatments with leaf moringa extract; irrigated at 60% of WCC (60% CC); irrigated at 60% of WCC plus treatments with leaf moringa extract (60% CC + LME); irrigated at 40% of WCC (40% CC); irrigated at 40% of WCC plus treatments with leaf moringa extract (40% CC + LME). Water loss was determined by weighing the pots every two days using an electronic weighing device (capacity 3.4 kg and resolution of 0.01 g, Orma, model BCE4200), and was calculated from the difference in weights (weight after irrigation, when drainage stopped, and weight before irrigating again). Two pots from each replication were measured. The amount of water manually added to each pot was

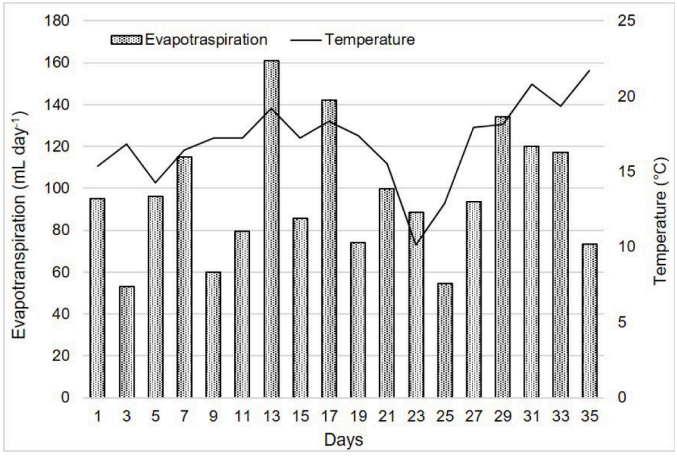

Fig. 1. Trend of evapotranspiration (mL day^{-1}) and average temperature (°C) during the experimental period.

1.7, 1.02, 0.5 L for 100% CC, 60% CC, and 40% CC respectively (Fig. 1). The electrical conductivity of the water was 0.85 dS m^{-1}. The trial started on 10 February 2022 and the growing period of the experiment lasted 35 days.

2.2. Meteorological data

The mean air temperature, relative humidity and global radiation were recorded on a data logger CR1000 (Campbell Scientific Ltd., Loughborough, UK) during the experimental periods. Mean temperatures ranged between 10 and 22 °C (Fig. 1). The relative humidity (RH) ranged between 32 and 96%.

2.3. Preparation of moringa oleifera extract

The *M. oleifera* leaf extract (LME) was prepared according to Toscano et al. (2021). The leaves of *M. oleifera* were shade-dried, and then finely grounded with a mill. The powder was mixed in distilled water (50 g in 200 mL). The blend was maintained for 48 h at 25 °C and then was filtered through filter paper Whatman No 1 and diluted in water 1:30, v/v (Zulfiqar et al., 2020). Tween 20 (0.05%) was used as a wetting agent.

The LME extract was analyzed, and its chemical constituents have been reported in Toscano et al. (2021).

2.4. Growth parameter measurement

At the end of the experiment, the substrate was removed from the roots, and six plants per treatment (three for each repetition) were divided in stems, leaves, flowers, and roots in order to measure the biometric parameters. After recording the fresh biomass (FW), the dry biomass (DW) was determined after drying the biomass at 70 °C to constant weight. The specific leaf area (SLA) was determined as the ratio of the leaf area to the leaf dry biomass. The total leaf number and leaf area were measured by leaf area meter (Delta-T Devices Ltd, Cambridge, UK).

2.5. Physiological parameters: Gas exchange, chlorophyll a fluorescence, and relative water content

For the measurement of gas exchange a CO_2/H_2O infrared gas analyzer (LCi, ADC Bioscientific Ltd., Hoddesdon, UK) was used. The measurements were effectuated at the end of the experiment in six plants per treatment (two plants for each repetition and three leaves per plant). Net photosynthetic rate ($A_{N=}$ μmol CO_2 m^{-2} s^{-1}), stomatal conductance ($g_{s=}$ mol m^{-2} s^{-1}), evapotranspiration (E= mmol m^{-2} s^{-1}), and Water Use Efficiency (WUE= μmol CO_2 m^{-2} s^{-1}/mmol H_2O) were registered.

At the same time, the chlorophyll *a* fluorescence was measured by a modulated chlorophyll fluorimeter OS1-FL (Opti-Sciences Corporation, Tyngsboro, MA). Each leaf was dark-adapted for 15 min using cuvette clips (Opti-Sciences Corporation, Tyngsboro, MA). The chlorophyll fluorescence was expressed as the F_v/F_m ratio, which indicates the maximal quantum yield of PSII photochemistry, where F_0= the minimum fluorescence, F_m = the maximal fluorescence of the dark-adapted state, and F_v = the variable fluorescence.

The relative water content (RWC) was determined by removing 30 discs of 10 mm in diameter from expanded leaves, and the fresh weights (FW) were obtained. The discs were immersed in distilled water for 24 h until reaching the turgid weight (TW). Subsequently, the discs were placed at 75 °C for 24 h to obtain the dry weights (DW). The RWC was calculated by the formula: RWC% = (FW − DW/TW − DW) * 100

2.6. Photosynthetic pigments

Chlorophyll and carotenoids content were measured based on the method described by Lichtenthaler et al. (1987). Leaf pigments (100 mg) were extracted by 99% methanol and incubated in the dark for 24 h at 4 °C. Quantification was performed by spectrophotometry (UV-1900i UV–VIS Spectrophotometer, 230 V, Shimadzu, Tokyo, Japan) and samples were read at 665.2 nm, 652.4 nm, and 470 nm. The amount of pigment contents was performed using the following formula:

$$\text{Chla} = 16.75_{A665.2} - 9.16_{A652.4}$$

$$\text{Chlb} = 34.09_{A652.4} - 15.28_{A665.2}$$

$$\text{Carotenoids} = (1000_{A470} - 1.63_{\text{Chla}} - 104.96_{\text{Chlb}})/221$$

2.7. Estimation of proline content

Ahmad et al. (2008) protocol was used for proline quantification using L-proline as the standard. Fresh leaves (1 g) were ground in nitrogen liquid. The samples were homogenized in 5 mL of 3% aqueous sulfosalicylic acid and centrifuged for 15 min at 14,000 *g* (Neya 10R, REMI, Mumbai, India). After centrifugation, the homogenate (2 mL) was added to the same volume of acetic acid and ninhydrin, blended and incubated (100 °C for 1 h). Then, the reaction was stopped in an ice bath, and the supernatant was extracted with 4 mL of toluene. The absorbance of the supernatant was recorded at 525 nm (UV-1900i UV–VIS Spectrophotometer, 230 V, Shimadzu, Tokyo, Japan).

2.8. Estimation of MDA content

Malondialdehyde (MDA) content was used according to Li et al. (2010). Leaf samples (0.5 g) was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) (w/v), and the homogenate was centrifuged at 5000 g for 10 min. The supernatant (2 mL) was mixed with the same quantity of 0.67% thiobarbituric acid. The reaction mixture was heated at 95 °C for 30 min and then centrifuged at 5000 g for 10 min. The MDA content was measured using the following formula: C (μmol/L) = 6.45 × (A532 − A600) − 0.56 × A450.

2.9. Antioxidant enzymes

Enzyme extract was prepared by 0.5 g of leaf homogenized in 4 mL of extraction buffer contain 50 mM potassium phosphate, 1 mM EDTA, 1% polyvinylpyrrolidone (w/v) (PVP), 1 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl (PMSF). The homogenate was centrifuged at 15,000 g for 30 min at 4 °C. The supernatant was collected and used to determine the catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD). The catalase activity was determined according to Aguilera et al. (2002); the reaction buffer contained 50 mM of potassium phosphate buffer (pH 7) and 150 μL of H_2O_2. The reaction was started with the addition of 20 μL of the extract and the decrease was registered at 240 nm for 2 min. The CAT activity was expressed as units of mg^{-1} protein. The glutathione peroxidase activity (GPX) was determined by Ruley et al. (2004) protocol. Enzyme extract and 17 mM H_2O_2 at the same quantity was homogenised with 2% guaiacol. Activity was measured by the increase in absorbance at 510 nm for 3 min. The activity of GPX was expressed as units of mg^{-1} protein. The superoxide dismutase activity (SOD) was measured by Giannopolitis and Ries (1977) protocol. The SOD activity was read at 560 nm. One unit of SOD was defined as the amount of enzyme added by 50% inhibition of NBT. The unit of SOD was expressed as units of mg^{-1} protein. All samples were read using a spectrophotometer (UV-1900i UV–VIS

Spectrophotometer, 230 V, Shimadzu, Tokyo, Japan). The protein content was determined by Bradford's method (1976).

2.10. Total phenol content (TPC) and sugars

Total phenolic content (TPC) was determined using Folin-Ciocalteu reagent. One g of fresh leaf sample was extracted with 10 mL of 50% acetone and incubated for 15 h at 20 °C.

One hundred microliters of extract were mixed to 0.5 mL of Folin-Ciocalteu reagent, 6 mL of distilled water and 1.5 mL of Na_2CO_3 (20%). After 120 min at Room Temperature, absorbance was measured at 765 nm. The TPC was expressed as gallic acid equivalent (mg g^{-1}).

Total sugars were measured by Yemm and Willis protocol (1954). Fresh leaves (1 g) were ground in nitrogen liquid and were extracted in 3 mL of distilled water; the samples were centrifuged at 3000*xg* for 15 min at room temperature (RT). Then, 1 mL of extract was mixed with 5 mL of anthrone solution (0.2 g in 100 mL of H_2SO_4), cooled in ice for 5 min and then mixed thoroughly. The samples were heated at 95 °C for 5 min and then cooled on ice. The absorbance was measured at 620 nm. The content of total soluble sugar was calculated using glucose as the standard.

2.11. Hormonal determination

The qualitative and quantitative analysis of phytohormones was carried out according to Gómez-Bellot et al. (2021). Briefly, 0.1 g of fresh leaves from 6 samples per treatment (2 samples per replicate) were crushed in a mortar with liquid nitrogen and stored at −80 °C. Then, they were vortexed with 0.5 mL 80% methanol/water (v/v) and incubated at 4 °C during 30 min and finally centrifuged at 15,000 rpm (20, 627 × g), at 4 °C for 15 min. The supernatant was kept in ice and then it was further extracted with 0.5 mL 80% methanol/water (*v/v*) after being incubated and centrifuged under the same conditions described above. Finally, both supernatants from the two previous extractions were passed through Chromafix C_{18} solid phase extraction cartridge (Macherey Nagel, Düren, Germany) (previously activated with 3 mL 80% methanol/water (*v/v*). The eluted sample was concentrated to dryness by the use of a rotary vacuum evaporator during approximately 3 h (Speedvac, Thermo, Waltham, MA, USA). Then, the dry residue was re-suspended with 200 μL de 20% metanol/water (*v/v*), sonicated for 8 min and filtrated through 0.45 μm polyethersulfone filter (Millipore) and finally quantificated by ultra-performance liquid chromatography (UPLC) coupled with a tandem quadrupole mass spectrometer (qMS/MS) equipped with an electrospray interface (ESI).

The separation of plant hormones was developed in accordance with Müller and Munné-Bosch (2011). Briefly, the UPLC system consisted of an Aquity UPLC™ System (Waters, Milford, MA USA) quaternary pump equipped with an autosampler. For the analysis of the extracts, a HALO™ C18 (Advanced Materials Technology, Inc., Wilmington, USA) column (2.1 × 75 mm, 2.7 μm) was used. Gradient elution was done with water and 0.05% glacial acetic acid (solvent A) and acetonitrile with 0.05% glacial acetic acid (solvent B) at a constant flow rate of 0.6 ml min^{-1}. The gradient profile was applied as follow: (t (min),% A): (0, 99), (2.20, 0), (2.40, 0), (2.60, 99), (3, 99). MS and MS/MS experiments were performed on an API 3000 triple quadrupole mass spectrometer (PE Sciex, Concord, Ont., Canada). Analyses were performed using Turbo Ionspray source in negative ion mode. Temperature was 400 °C, nebulizer gas (N2) 10 (arbitrary units), curtain gas (N2) 12 (arbitrary units), collision gas (N2) 4 (arbitrary units) and the capillary voltage was −3.5 kV. The mass spectrometer was operated in multiple reaction mode (MRM) due to their high selectivity using precursor-to-product ion transitions because many compounds could present the same nominal molecular mass or peaks can overlap.

2.12. Aminoacid determination

A homogeneous (50 mg) sample was weighed into sample tube containing 10 mL of 0.1% (v/v) formic acid in water:methanol (80:20) (v/v). The mixture was shaken with vortex for 5 min, then immediately centrifuged at 4 °C at 4000 rpm for 15 min. The supernatant was passed through 0.2 μm PTFE membrane filter and 1 μL of sample was injected to UPLC–MS/MS (Nimbalkar et al., 2012).

The UPLC–MS/MS instrument consisted of a Waters (Milford, MA, USA) Acquity Ultra Performance LC with a Waters binary system manager coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer equipped with electro spray ionization (ESI) probe. Separation was achieved using C18 column (Acquity UPLC BEH C18 100 mm 2.1 mm, 1.7 lm particle size) according to Kıvrak et al. (2014). A 1 μL injection volume was used. The solvent system consisted of 0.5% aqueous formic acid (A) and methanol/water (50:50, v/v) containing 0.5% formic acid (B). The ESI source was used in positive mode by multiple reaction monitoring (MRM) mode with the following conditions: The ion source capillary voltage was 0.8 kV; cone voltage was 20 V, desolvation temperature was 400 C, pressure of nebulizer gas was 7 bar. The collision gas was argon. The cone voltage varied from 17 to 27 V, depending on amino acid investigated. Data analysis and quantitation were executed using the Waters MassLynx and TargetLynx software.

2.13. Statistical analysis

The trial was tested in a randomized complete design with three replicates per treatment. The results were analysed by one way and two-way ANOVA by CoStat version 6.311 (CoHortSoftware, Monterey, CA, USA). Interaction effects were calculated using Tukey's test at a 5% level of significance. The principal component loading plot and scores of PCA were performed using Minitab 16, LLC.

3. Results

3.1. Evapotranspiration

Fig. 1 shows the trend of evapotranspiration (L day^{-1}) in the 100% CC treatment during the experimental period. The amount of water manually added to each pot was 1.74, 1.04, and 0.70 L, respectively, for 100% CC, 60% CC and 40% CC. The trend of evapotranspiration appeared to be directly related to the temperatures reached and varied between 56 and 161 mL d^{-1}.

3.2. Biomass and leaf area

The growth of petunia was significantly modified by treatments (Table 1).

The total dry biomass was influenced by water ($p < 0.001$) and biostimulant ($p < 0.01$) treatments, but not their interaction ($p > 0.05$) (Table 1). The shoot dry biomass showed significant differences in all treatments (Table 1). The sprayed with moringa leaf extract (*T*) increased this parameter in different irrigation conditions, as observed when we compare 100% CC with 100% CC+LME and 40% CC with 40% CC+LME with an increase by 29% and 26% respectively (Fig. 2A).

The root-to-shoot ratio was influenced by biostimulant ($p < 0.01$) and showed an effect of interaction ($p < 0.01$) (Table 1). The root-to-shoot ratio, in fact, increased in petunia plants grown under full irrigation conditions (100% CC) (Fig. 2B). Leaf number was significantly reduced under 40% CC (by 40%) compared with the 100% CC ($p < 0.001$) (Table 1); the treatment with moringa had alleviated the effect of drought stress in 40% CC+LME, increasing the leaf number by 23% compared to 40% CC (Fig. 2C). The total leaf area showed an increase for the effect of biostimulant treatment. In 100% CC +LME and in 40% CC +LME was observed an increase by 23% and 14% respectively (Fig. 2D). The SLA was affected only by water treatments ($p < 0.01$).

Table 1
Mean effects of water stress treatments and biostimulant treatment (LME) on total and shoot biomass, root/shoot ratio (R/S), leaf number, total and unit leaf area of potted petunia plants during the growing period. Plants were irrigated every two days. Three water stress treatments were considered: irrigated at 100% CC,; irrigated at 60% CC, and irrigated at 40% CC. The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water.

Water treatments	Biostimulant treatment	Total biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	R/S ratio (g^{-1})	Leaf number (n. plant^{-1})	Total leaf area (cm^2 plant^{-1})	Flower number (n. plant^{-1})	SLA (cm^2 plant^{-1})
100% CC		5.25±0.34a	4.13±0.36a	0.29 ±0.04a	160.58±10.30a	358.30 ±27.03a	16.20±0.82a	163.99 ±7.66a
60% CC		4.02±0.11b	3.31±0.08b	0.22 ±0.01a	138.51±3.17b	207.47 ±3.44b	18.75±0.40a	128.54 ±3.43b
40% CC		2.71±0.27c	2.24±.0.25c	0.22 ±0.02a	114.67±6.15c	217.61 ±7.55b	10.85±1.20b	148.80 ±7.11ab
	C	3.62±0.37b	2.85±0.28b	0.27 ±0.03a	127.17±6.79b	241.49 ±16.47b	13.00±0.85b	151.80 ±14.13a
	LME	4.37±0.42a	3.61±0.35a	0.21 ±0.01b	148.67±8.79a	280.77 ±33.91a	16.33±0.96a	142.42 ±5.39a
Significance								
Water treatments (*W*)		***	***	ns	***	***	**	**
Biostimulant Treatments (*T*)		**	***	**	***	***	**	ns
W x *T*		ns	***	**	***	**	ns	ns

Values are means for the main effects of water treatments (W) and biostimulant treatment (T).
The statistical analysis was two-way ANOVA; ns not significant;
* significant at $p < 0.05$;
** significant at $p < 0.01$;
*** significant at $p < 0.001$. The values in the same column followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test).

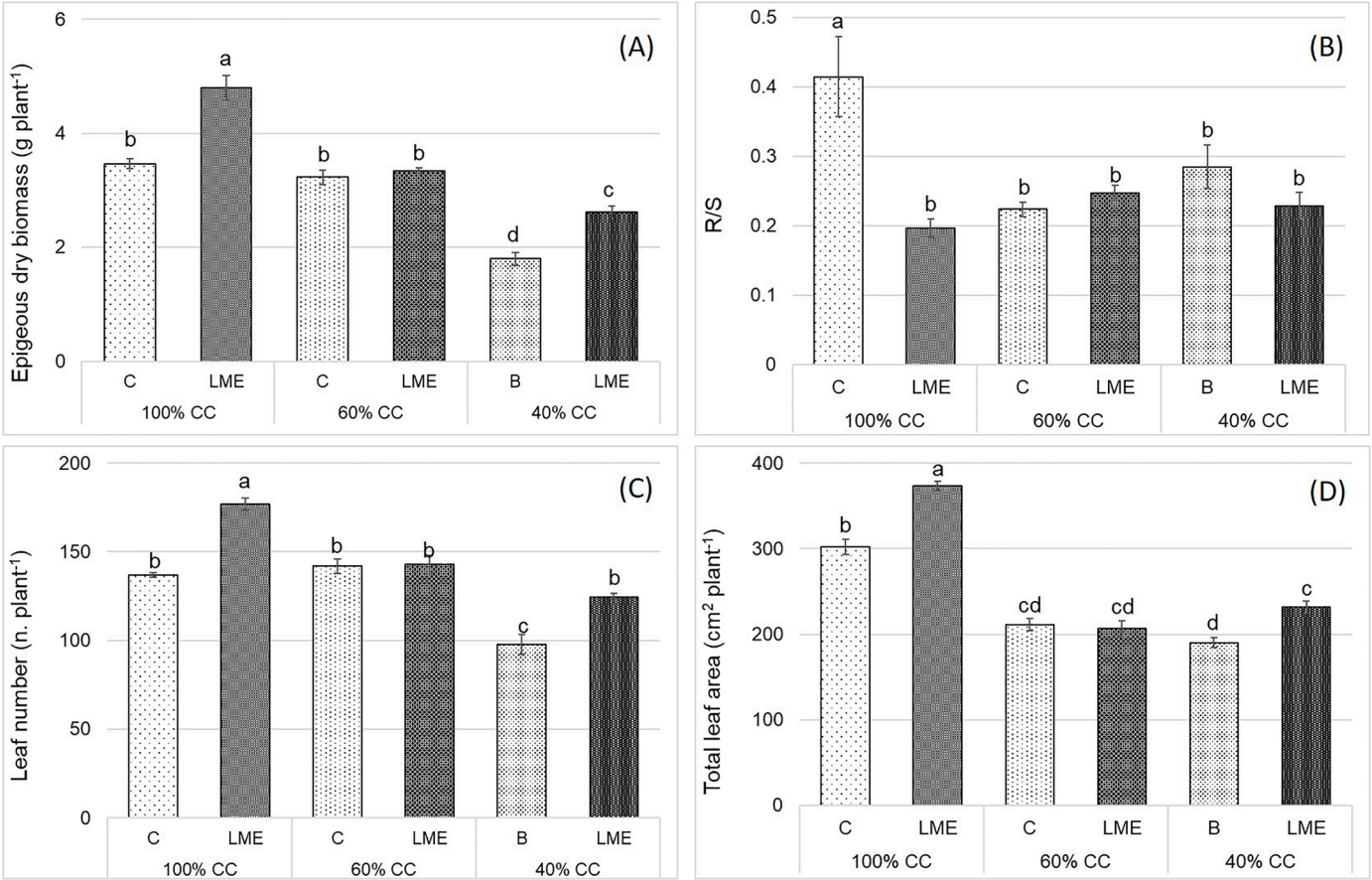

Fig. 2. Interactions between water treatments and biostimulant treatment in petunia plants: epigeous dry biomass (A), root/shoot ratio (R/S) (B), leaf number (C) and total leaf area (D). The vertical bars indicate mean ± S.E. ($n = 3$). Columns denoted with the same letters are not significantly different, as determined by Tukey's test ($p < 0.05$).

The flower number was influenced by water ($p < 0.01$) and biostimulant ($p < 0.01$) treatments, but not their interaction ($p > 0.05$) (Table 1). The flower number showed significant differences in 40% CC compared to the other treatments (Table 1). The flower numbers showed an increase for the effect of biostimulant treatment ($p < 0.01$) with an increase by 25% (Table 1). No significant effects of interaction were observed.

The SLA showed significant differences by effect of water deficit treatments (W, $p < 0.01$).

3.3. Plant physiological measurements

Net photosynthesis (A_N) was significantly affected by water stress and interaction of W x T (Fig. 3a). Under severe drought stress conditions (40% CC), A_N reduced considerably (by 22%) compared to control (100% CC) plants. The 60% CC+LME showed the same value compared to the 60% CC (Fig. 3A). The gs was significantly affected by water stress and showed an interaction with biostimulant treatment; in fact, a higher value was observed for 100% CC+LME with an increase by 10%. The lowest values were observed in both 40% CC and 40% CC+LME compared to control plants with a reduction by 50% (Fig. 3B).

No significant effects of water, biostimulant treatment, and their interaction were observed for E (Fig. 3C).

The WUE showed a significant increase in 60% CC+LME, 40% CC, and 40% CC+LME compared to the rest of the treatments (Fig. 3D).

The minimum fluorescence and the maximum fluorescence did not show any significant differences (Table 2). The maximum quantum efficiency of PSII (Fv/Fm) showed a significant change in response to drought stress. In 40% CC water deficit treatment, the value of Fv/Fm reached the lowest value (0.78) at the end of the experiment (Table 2).

The RWC varied with the drought stress treatments, but not with biostimulant treatment and their interaction. The maximum value was observed for the 100% CC (75.8%) and the minimum in the severe water treatment (53.5% in the 40% CC) (Table 2).

3.4. Chlorophyll and carotenoid content

The Chl and carotenoid content exhibited a different pattern in relation to water and biostimulant treatment (Table 3).

No effect of drought stress treatments was observed on Chl a ($p > 0.05$), whereas, Chl a content increased for the effect of LME treatment ($p < 0.05$). Significant effects of water and biostimulant treatment were observed on Chl b content (W, $p < 0.05$; T, $p < 0.001$). As a result, total Chl content followed the same pattern as Chl a (Table 3). The interactive effect of water × treatment was not significant for all chlorophyll parameters ($p > 0.05$).

Carotenoids were accumulated in a larger amount in 100% CC and 60% CC (0.13 mg g^{-1} FW, 0.12 mg g^{-1} respectively). Carotenoid content increased for the effect of LME treatment ($p < 0.05$). No interaction (W x T) was observed for this parameter ($p > 0.05$) (Table 3).

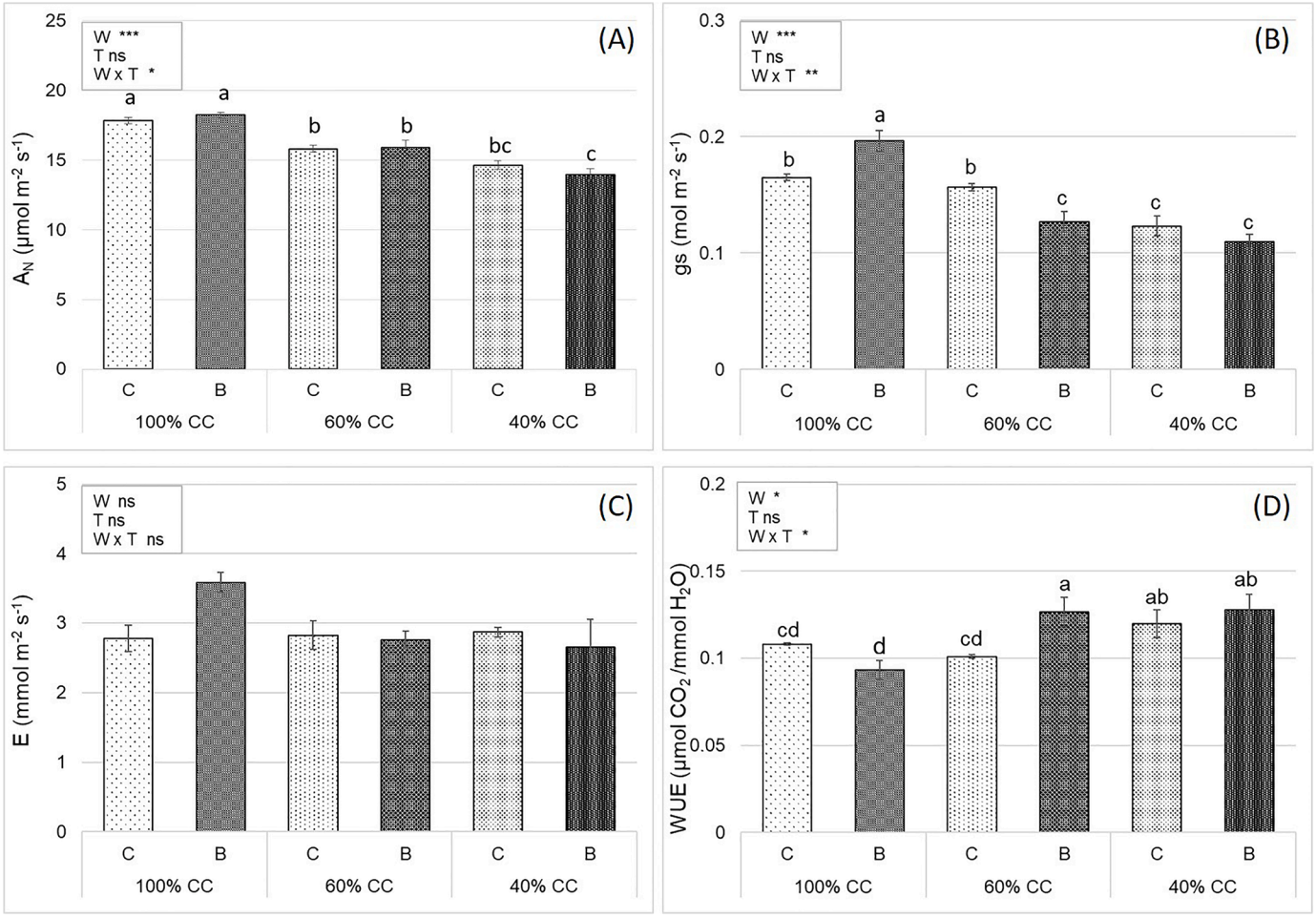

Fig. 3. Interactions between water treatments and biostimulant treatment in petunia plants: net photosynthesis (A_N) (A), leaf conductance (gs) (B), transpiration rate (E) (C), and water use efficiency (WUE) (D). Plants were irrigated every two days. Three water stress treatments were considered: irrigated at 100% CC (100% CC), irrigated at 60% CC (60% CC), and irrigated at 40% CC (40% CC). The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water until dripping. Mean are values ± standard error (S.E) ($n = 6$). Different letters indicate significant differences among the treatments as determined by Tukey's test ($p < 0.05$).

Table 2
Effects of water stress treatments and biostimulant treatment (LME) on minimum fluorescence (F0), maximum fluorescence (Fm), chlorophyll *a* fluorescence (Fv/Fm), and Relative Water Content (RWC) of petunia potted plants. Plants were irrigated every two days. Three water treatments were considered: irrigated at 100% of container capacity (100% CC), irrigated at 60% CC, and irrigated at 40% CC. The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants control were sprayed with distilled water.

Water treatments	Biostimulant treatment	F0	Fm	Fv/Fm	RWC
100% CC		311.3 ± 34.5a	1672.5 ± 27.4a	0.81±0.0a	75.8 ± 1.5a
60% CC		319.3 ± 58.1a	1662.3 ± 50.3a	0.80±0.0a	56.9 ± 1.4b
40% CC		337.3 ± 29.7a	1687.5 ± 21.8a	0.78±0.0b	53.5 ± 0.4b
	C	318.6 ± 9.0a	1637.6 ± 31.7a	0.80±0.0a	58.2 ± 1.6a
	LME	326.8 ± 6.3a	1710.7 ± 31.0a	0.80±0.0a	58.1 ± 2.0a
Significance					
Water treatments (W)		ns	ns	**	***
Biostimulant Treatments (T)		ns	ns	ns	ns
W x T		ns	ns	ns	ns

Values are means for main effects of water treatments (W) and biostimulant treatment (T).
The statistical analysis was two-way ANOVA;ns not significant;
** significant at $p < 0.01$;
*** significant at $p < 0.001$. The values in the same column followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test).

The chlorophyll a/b ratio showed that biostimulant treatment provided the highest value ($p < 0.05$). The relationship between total chlorophyll and carotenoids expressed as a ratio showed a significant difference by water stress ($p < 0.001$) and biostimulant treatments ($p < 0.001$) but not their interaction (Table 3).

3.5. Proline content and MDA content

The amount of leaf proline content was affected by drought stress treatments, biostimulant treatment, and their interaction ($p < 0.001$). In fact, the lowest amount of proline (~11 nmol g^{-1} FW) was observed in control plants (100% CC and 100% CC + LME); an increase in 60% CC (~24 nmol g^{-1} FW) and 60% CC +LME (~34 nmol $^{-1}$ FW) was observed. The highest quantity in 40% CC control plants (~108 nmol g^{-1} FW) and 40% CC + LME (~56 nmol g^{-1} FW) was founded (Fig. 4a).

At the end of the experiment, the MDA content increased in the 60% CC and 40% CC compared with the control plants (Fig. 4b). In particular, the highest value was observed for 40% CC plants (~18 nmol g^{-1} FW). The LME biostimulant application decreased lipid peroxidation (MDA) of petunia under severe water treatments (40% CC+LME), reducing the value at 15 nmol g^{-1} FW respect to 40% CC.

3.7. Enzyme activity and protein content

Biostimulant treatment decreased the GPX activity in 100% CC plus LME (100% CC + LME) (by 20%, Fig. 5A) while no significant differences were observed in the other water treatments. The CAT activity was influenced only by biostimulant treatments; in 60% CC +LME a reduction compared to 60% CC was observed (Fig. 5B). Protein content was significantly affected by water stress, biostimulant treatments, and their interaction (Fig. 5D). An increase was observed in relation to the water stress conditions. Under moderate and severe water stress (60% CC and 40% CC) LME limited the increase.

3.8. Total phenol compound (TPC) and sugars

The TPC showed significant differences in relation to the drought stress treatments ($p < 0.001$). The biostimulant treatment did not exert

Table 3
Effects of water stress treatments and biostimulant treatment (LME) on Chl*a*, Chl*b*, Chl*a*+*b*, Car, a/b ratio, and Car/Chl*a*+*b* on petunia potted plants. Plants were irrigated every two days. Three water treatments were considered: irrigated at 100% of container capacity (100% CC), irrigated at 60% CC, and irrigated at 40% CC. The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water.

Water treatments	Biostimulant treatment	Chl*a* (μg mg^{-1} FW)	Chl*b* (μg mg^{-1} FW)	Chl*a*+*b* (μg mg^{-1} FW)	Car (μg mg^{-1} FW)	Chl*a*/Chl*b* (μg mg^{-1} FW)	Car/ Chl*a*+*b*
100% CC		0.61±0.06a	0.28±0.05b	0.89±0.10a	0.13±0.01a	2.30±0.20a	0.16 ±0.02a
60% CC		0.66±0.03a	0.33±0.04ab	0.99±0.07a	0.12±0.01a	2.07±0.18ab	0.12 ±0.01b
40% CC		0.61±0.03a	0.38±.0.06a	1.00±0.08a	0.08±0.01b	1.79±0.25b	0.08 ±0.02c
	C	0.57±0.02b	0.23±0.02b	0.80±0.03b	0.12±0.01a	2.47±0.11a	0.16 ±0.01a
	LME	0.68±0.03a	0.43±0.03a	1.11±0.05a	0.10±0.01b	3.37±0.11b	0.09 ±0.01b
Significance							
Water treatments (W)		ns	*	ns	**	*	***
Biostimulant Treatments (T)		*	***	***	*	***	***
W x T		ns	ns	ns	ns	ns	ns

Values are means for main effects of water treatments (W) and biostimulant treatment (T).
The statistical analysis was two-way ANOVA; ns not significant;
* significant at $p < 0.05$;
** significant at $p < 0.01$;
*** significant at $p < 0.001$. The values in the same column followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test).

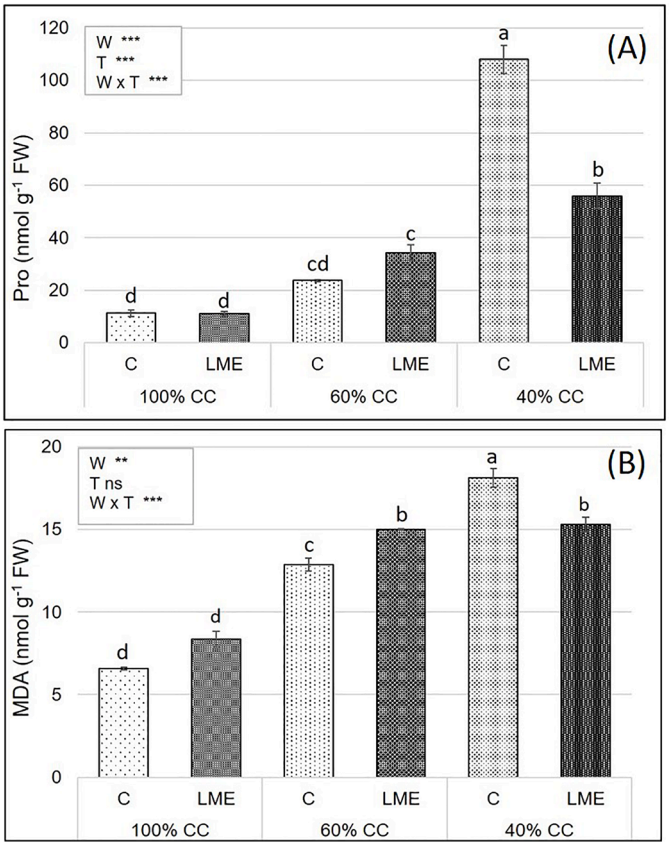

Fig. 4. Effects of water treatments and biostimulant treatment on proline content (Pro) (A) and MDA content (B) of petunia plants at the end of the experimental period. Plants were irrigated every two days. Three water stress treatments were considered: irrigated at 100% CC (100% CC), irrigated at 60% CC (60% CC), and irrigated at 40% CC (40% CC). The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water until dripping. Mean are values ± standard error (S.E) ($n = 3$). Different letters indicate significant differences among the treatments as determined by Tukey's test ($p < 0.05$).

any effect on this parameter (*T*, $p > 0.05$), while the interactive effect with the treatment (*W* x *T*, $p < 0.001$) was significant (Fig. 6A). The value was significantly increased in the 100% CC + LME (by 23%) compared to 100% CC plants whereas the treatment with LME affected the TPC in more severe water treatments with a reduction by 30% (Fig. 6A).

Total soluble sugars in the leaf tissues were significantly affected only by drought treatments ($p < 0.001$). In particular, the values were significantly increased in the 60% CC (by 41%) and 40% CC (31%) compared to control plants (100% CC) (Fig. 6B).

3.8.1. Hormones and aminoacids

Several hormones were identified in leaves of petunia: indoleacetic acid (IAA), jasmonic acid (JA), abscisic acid (ABA), salicylic acid (SA) (Fig. 7), gibberellic (GA_3) (data not show) and indolebutyric acid (IA) (data not show).

Some of them (IAA and JA) were modified by the water treatments but not by biostimulant treatment (*T*, $p > 0.05$), and their interaction (*W* x *T*, $p > 0.05$). A higher IAA concentration was observed in 60% CC compared with those irrigated with 100% CC (Fig. 7A). The jasmonic acid showed an increase as the water deficit progressed, with an increase by 51% and 71% respectively for 60% CC and 40% CC (Fig. 7B).

The ABA content showed significant differences in relation to the drought stress treatments (*W*, $p < 0.05$) and biostimulant treatment (*T*, $p < 0.001$), while the interactive effect with the treatment was not significantly (*W* x *T*, $p > 0.05$) (Fig. 7C). Under water deficit treatments, the ABA content of petunia reached its highest value (10.6 ng^{-1} g) in the 60% CC and in 40% CC (9.2 ng^{-1} g).

The SA content showed significant differences in relation to the water deficit treatments (*W*, $p < 0.05$), biostimulant treatment (*T*, $p < 0.001$), and their interaction (*W* x *T*, $p < 0.001$) (Fig. 7D). The highest value (47.6 ng g^{-1}) was found in 100% CC and in 60% CC compared with the other treatments (Fig. 7D).

There was a significant increase in the free amino acid contents in petunia leaves as water deficit increased (Table 4). The highest content for almost all free amino acids was observed in the most stressed treatment (40% CC and 40% CC + LME).

3.9. Analysis of PCA

PC1 and PC2 accounted for 48.6% and 12.6% of the variance, respectively (Fig. 8). Physiological (A_N, gs, RWC, E, Fv/Fm) and morphological (EB, TB, SLA, R/S, LA, LN) parameters and TPC were key factors with negative scores in PC1. By contrast, WUE, F0, Fm, enzyme activity, MDA, Pro, Chl *a, b* and total, ABA, JA, IA, and II, and all amino acids except the valine content were key factors with positive scores. All morphological and physiological parameters, CAT and TPC, Chl content, IA, GA_3, and amino acids (Ala-Arg, Asp, Glu-acid, Gly, Iso, Leu, Lys, Phen, Serm, Thre, and Meth) were positive scores with PC2. The other parameters were negative scores with PC2. The treatments of the petunia plants under drought stress and well-watered conditions were completely separated (Fig. 9). This pattern could be explained by different physiological and biochemical reactions between the drought stress, alone and in combination with LME, and control plants.

4. Discussion

Water deficit often affects bedding plants in urban areas and in particular in the Mediterranean area causing negative effects in particular on the esthetic value (Álvarez et al., 2013). It is therefore needed to individuate species that are capable to tolerate water scarcity without losing their ornamental values (Chyliński et al., 2007). To carry out the selection of drought-resistant plants, an evaluation of the physiological and biochemical response appears to be an efficient approach (Rafi et al., 2019). To describe the tolerance of plants to drought stress the growth reduction in stress conditions in interaction with physiological and biochemical parameters has been used (Toscano et al., 2016).

In relation to the possible role of different biostimulants to improve abiotic stress have been investigated different plant species. Our experiment determined the irrigation level thresholds for petunia plants to attain satisfying growth and visual quality. The results showed that the plants grow up to 40% CC, however treated with the biostimulant, showed good resistance to water stress by activating various physiological and biochemical mechanisms. The drought water stress reduced growth parameters compared with full irrigation and the moringa foliar application promoted the growth parameters in both control and water deficit conditions. LME as a natural and eco-friendly biostimulant has been useful to improve the growth and productivity characteristics of numerous species grown in normal conditions (Rehman et al., 2015; Ashraf et al., 2016; Elzaawely et al., 2016; Nasir et al., 2016) and even with saline and drought stress conditions (Rady et al., 2013; Yasmeen et al., 2013a; Howladar 2014; Hanaf, 2017). Moringa extracts are considered a suitable alternative source of inorganic fertilizers due to their high content of micro and macro mineral nutrients, protein, and essential amino acids, which supplement the nutritional requirements of crops (Yasmeen et al., 2013b). In our results, a significant reduction of epigeous dry matter, number and total leaf area was observed with the increase of water deficit treatments, indicating that water stress inhibited plant growth. The biggest reduction was observed in 40% CC, but the biostimulant mitigated the effect of water stress with an increase in these parameters. This increase can be attributed to plant hormones

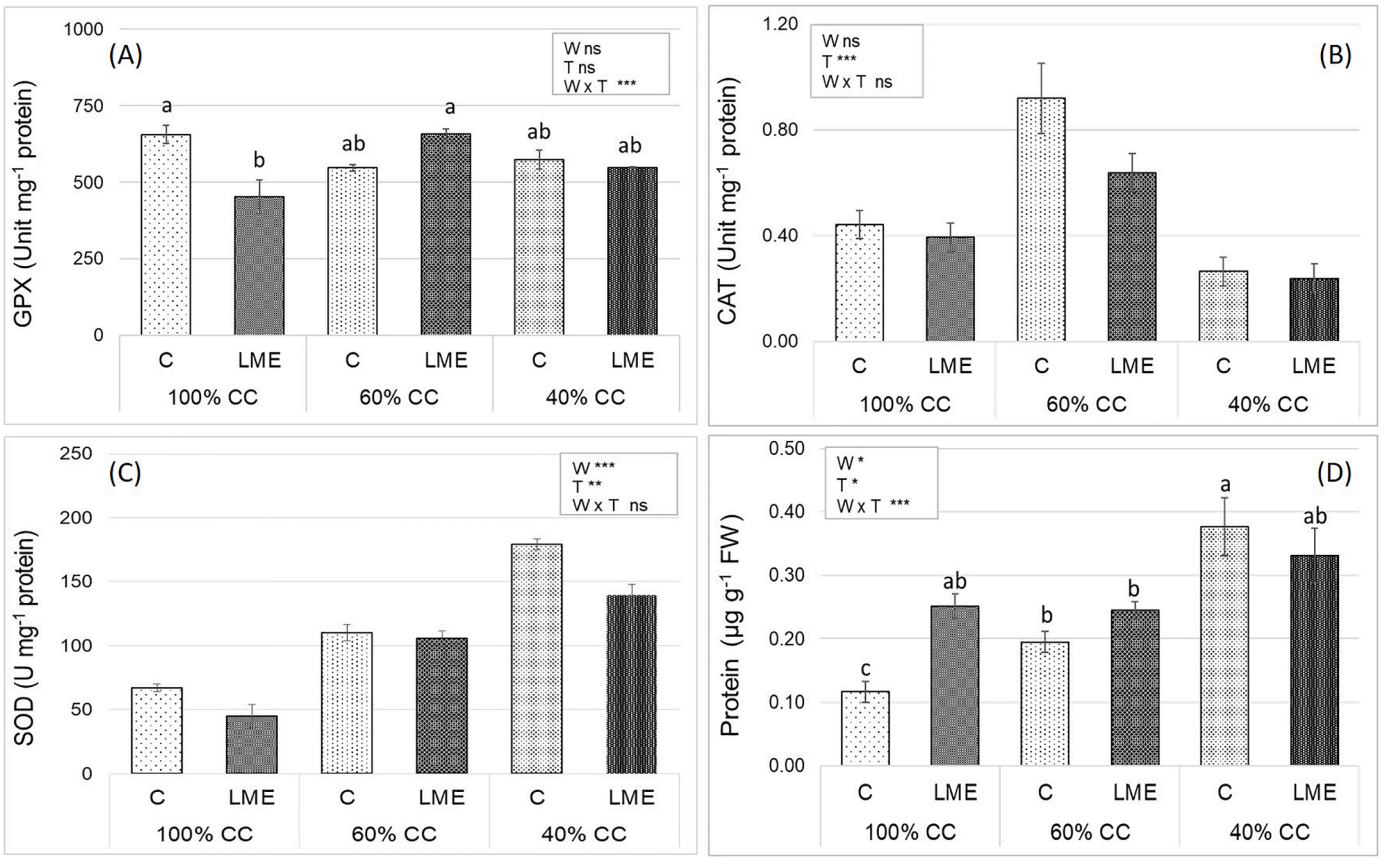

Fig. 5. Effects of water treatments and biostimulant treatment on Glutatione peroxidase (GPX) (A), Catalase (CAT) (B), Superoxide dismutase (SOD) (C), and Protein content (D) of petunia plants at the end of the experimental period. Plants were irrigated every two days. Three water stress treatments were considered: irrigated at 100% CC (100% CC), irrigated at 60% CC (60% CC), and irrigated at 40% CC (40% CC). The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water until dripping. Mean are values ± standard error (S.E) ($n = 3$). Different letters indicate significant differences among the treatments as determined by Tukey's test ($p < 0.05$).

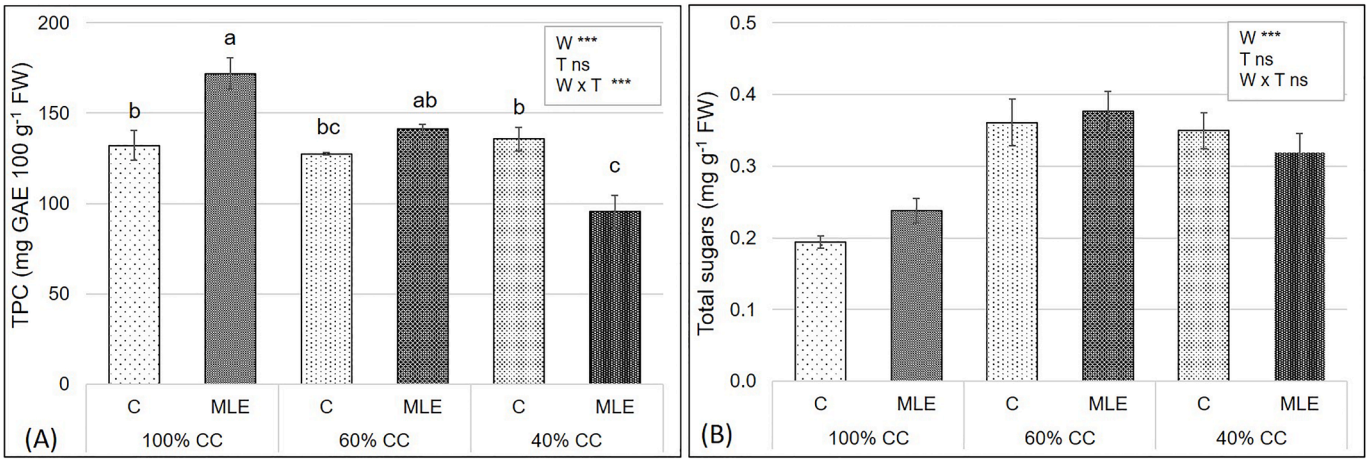

Fig. 6. Effects of water treatments and biostimulant treatment on leaf total phenol compound (TPC) (A) and total sugars (B) of petunia plants at the end of the experimental period. Plants were irrigated every two days. Three water stress treatments were considered: irrigated at 100% CC (100% CC), irrigated at 60% CC (60% CC), and irrigated at 40% CC (40% CC). The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water until dripping. Mean are values ± standard error (S.E) ($n = 3$). Different letters indicate significant differences among the treatments as determined by Tukey's test.

contained in the Moringa leaf extract, which stimulate every stage of plant growth and development. The leaves of moringa are rich source of cytokinin, antioxidants, K, Ca and micronutrients, which have a plant growth promoting capabilities and regularly applied as exogenous plant growth enhancers (Abdalla, 2013). This behavior was observed by Zeljkovićet al. (2021); these authors showed that in the case of annual ornamental seedlings, the weight of the above-ground parts can also be increased by using biostimulants.

The irrigation treatments in our study had a significant effect on the aerial biomass accumulation, but no significant effects on root biomass,

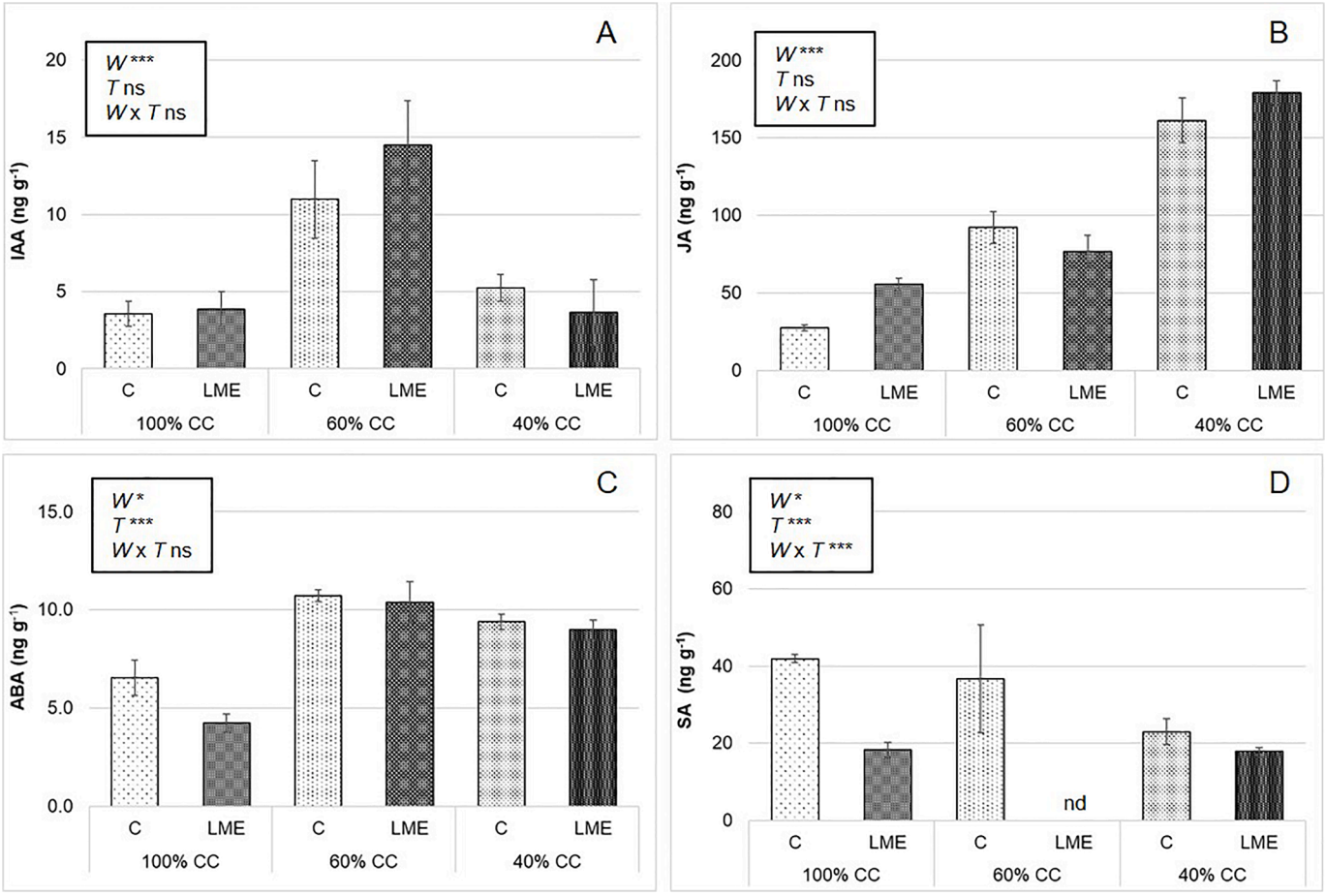

Fig. 7. Effects of water treatments and biostimulant treatment on indoleacetic (IAA) (A), jasmonic (JA) (B), abscisic (ABA) (C), and salicylic acid (SA) (D) of petunia plants at the end of the experimental period. Plants were irrigated every two days. Three water stress treatments were considered: irrigated at 100% CC (100% CC), irrigated at 60% CC (60% CC), and irrigated at 40% CC (40% CC). The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water until dripping. Mean are values ± standard error (S.E) ($n = 4$). Different letters indicate significant differences among the treatments as determined by Tukey's test.

indicating that shoots and roots respond differently to water stress (Sánchez-Blanco et al., 2009). More plant species increase their root biomass to enhance water uptake (Jaramillo et al., 2013) and hence maintain the plant water status and guarantee photosynthesis in water stress conditions. In the nursery stage of bedding plant cultivation, this response may not be possible because both root growth and available water are limited due to the small substrate volume.

We observed a significant increase in leaf area due to LME application in the most stressed plants, which is in accordance with the reports of Alì et al. (2018), using a commercial extract of the brown algal *(Ascophyllum nodosum)*. High plant growth, and therefore a greater leaf area development in the more stressed plants, might be due to growth-promoting hormones in LME, which may have the potential to trigger a higher plant growth rate. Drought stress reduced plant growth and was correlated with a decrease in photosynthesis activity and plant water status (Da Silva et al., 2022). A gradual reduction in gas exchange was found in our study following water stress treatments, which had been described in some studies on ornamental plants (Niu and Rodriguez 2009; Álvarez and Sánchez-Blanco 2013). The results of the current study showed that water stress treatments lead to a reduction of net photosynthesis, but the moringa application increased the activity in the treatment of 60% CC. Our results suggest that LME could stimulate the plant performance by keeping open stomata, maintaining photosynthesis, source-sink relations (growth), and thus protecting from possible photoinhibition/photooxidation effects. The gs values were also reduced by water stress; it suggested the adaptive and efficient control of transpiration and represents a mechanism to cope the drought stress, especially during high transpiration periods, by limiting water loss, as has been observed in other species (Hessini et al., 2008; Álvarez and Sánchez-Blanco 2013). Moreover, high water use efficiency (WUE, μmol CO_2 mol^{-1} H_2O), which is defined as the relation of biomass production to water consumption, is an effective index to describe the stability between photosynthetic activity and water consumption due to either environmental acclimation or genetic adaptation (Valdecantos et al., 2011). In our study, water stress affects WUE. This finding indicates that petunia plants could efficiently use water resources under moderate and severe drought stress conditions. WUE improvement is mainly due to the accumulation of dry matter through less water consumption thanks to the stomate closure and low transpiration.

Under water stress conditions, RWC is one of the most reliable tools for predicting the physiological traits of plants (Chaturvedi et al., 2019). RWC indicates plant water status, which is considered one of the most meaningful indices of dehydration tolerance (Antonić et al., 2016). Our results show that deficit irrigation reduces RWC, but LME application has not improved it under stress conditions. This is probably due to that LME increased the osmoprotectant concentrations (i.e., total soluble sugars and proline), which ultimately helps in maintaining better water balance in the plants. Moreover, the results showed that there is a negative and significant correlation between RWC and proline accumulation ($r = -0.80$). Decreasing RWC under stress conditions leads to the accumulation of ABA and reactive oxygen species (ROS) that may act as signaling molecules in root tissue. Accumulation of ABA under water

Table 4
Effects of water stress treatments and biostimulant treatment (LME) on amino acids (μg 100 mg^{-1}) on leaf petunia potted plants. Plants were irrigated every two days. Three water treatments were considered: irrigated at 100% of container capacity (100% CC), irrigated at 60% CC, and irrigated at 40% CC. The plants were sprayed every 10 days after transplant with moringa leaf extract (LME), and the control plants were sprayed with distilled water.

Treatments	Ala	Arg	Asn	Asp	Glu	Gln	Gly	His	Ile	Leu	Lys	Phe	Ser	Thr	Trp	Tyr	Val	Met	Cys	Total
100% CC C	20.7	1.3	0.0	4.6	1.1	0.0	11.6	0.6	63.6	93.0	1.5	8.8	2.7	2.5	7.6	6.3	55.7	3.4	0.0	291.2
	±1.6c	±0.3c	±0.0b	±0.5c	±0.3c	±0.0e	±2.2ab	±0.1b	±4.9c	±7.1c	±0.5c	±0.7c	±0.9c	±0.1c	±1.0b	±0.5c	±9.3ab	±0.3c	±0.0b	±5.3c
100% CC	23.8	1.9	0.0	3.9	2.0	9.2	9.4	0.8	72.7	97.2	2.3	14.5	4.2	3.1	11.4	5.8	73.3	3.4	0.0	346.2
LME	±0.4c	±0.1bc	±0.0b	±0.3bc	±0.1abc	±0.2d	±1.7ab	±0.0b	±3.7c	±1.9c	±0.0bc	±1.4bc	±0.4bc	±0.0bc	±2.3b	±0.3c	±6.7ab	±0.1c	±0.0b	±4.3c
60% CC C	33.3	4.8	0.0	9.0	1.4	32.9	19.1	2.4	121.7	172.3	4.3	23.1	8.9	5.4	37.2	10.7	24.8	6.6	0.0	529.7
	±0.9b	±0.3a	±0.0b	±0.3ab	±0.2bc	±0.7c	±0.6b	±0.1b	±3.2ab	±4.8ab	±0.1a	±1.0abc	±0.2ab	±0.3abc	±2.8a	±1.0abc	±1.1b	±0.4bc	±0.0b	±11.3b
60% CC	48.2	4.1	0.0	10.0	3.4	32.9	15.6	1.9	114.8	171.6	4.5	26.1	9.9	6.0	35.6	9.7	61.9	8.1	0.2	621.7
LME	±0.9a	±0.4ab	±0.0b	±0.8a	±0.6ab	±1.0c	±2.9ab	±0.2b	±1.6b	±3.7ab	±0.5a	±1.5ab	±0.7a	±0.4ab	±1.5a	±0.2bc	±15.8ab	±1.6ab	±0.0a	±41.0b
40% CC C	35.7	4.3	2.2	8.4	4.0	113.2	17.0	6.0	121.9	170.6	3.8	30.7	10.1	8.4	49.9	13.9	89.8	7.2	0.1	730.7
	±2.4b	±1.2ab	±1.5b	±2.2ab	±0.9a	± 7.8b	±3.4ab	±1.4a	±4.0ab	±2.1b	±0.4ab	±5.3a	±2.2a	±1.7a	±6.5a	±0.8ab	±17.8a	±0.4abc	±0.0a	±30.4a
40% CC	32.2	5.3	8.8	8.0	0.1	136.3	20.3	6.1	136.2	194.0	3.7	29.3	9.5	6.9	44.5	15.7	56.0	11.0	0.1	749.4
LME	±1.2b	±0.2a	±3.0a	±1.1ab	±0.1c	± 4.3a	±2.4a	±0.3a	±6.1a	±7.5a	±0.3ab	±6.0ab	±0.7a	±0.3a	±2.0a	±2.7a	±14.7ab	±1.4a	±0.0a	±23.6a
Significance	***	***	**	***	***	***	*	***	***	***	***	**	***	***	***	***	*	***	***	***

Abb.: Alanine = Ala; Arginine = Arg; Asparagine = Asn; Aspartic acid = Asp; Glutamic acid = Glu; Glutamine = Gln; Glycine = Gly; Histidine = His; Isoleucine = Ile; Leucine = Leu; Lysine = Lys; Phenylalanine = Phe; Serine = Ser; Threonine = Thr; Tryptophan = Trp; Tyrosine = Tyr; Valine = Val; Methionine = Met; Cysteine = Cys; 4-OH Hydroxy proline = Hyp. The statistical analysis was one-way ANOVA; * significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p< 0.001$.

stress conditions stimulates the production of proline synthesizing enzymes (Pál et al., 2018), therefore, the RWC reduction can be considered the first-factor stimulating proline synthesis.

In our study the chlorophyll content under water stress was unchanged; this is according to Rosales-Serna et al. (2004) whose reported that the maintenance of high chlorophyll content might be a physiological adaptation mechanism of plants under drought stress (Rosales-Serna et al., 2004). Application of LME is reported to enhance the chlorophyll content. The LME components may be acted also to induce more biosynthesis of leaf photosynthetic pigments due to its richness content of mineral nutrients and phytohormones, increasing chlorophyll content (Desoky et al., 2018).

One of the principal objectives of this experiment was to know individually the modifications in the accumulation of specific compounds in relation to water stress and the biostimulant application to improve drought tolerance. Increases in drought tolerance have been correlated with the capacity to accumulate protective compounds, including amino acids and carbohydrates (Oraee and A. Tehranifar, 2020). During water stress conditions, the accumulation of some molecules supports plant growth. Proline plays many other functions, as 'osmoprotectant,' directly stabilizing proteins, membranes and other subcellular structures, scavenging free radicals, or balancing the cell redox status under stress conditions (Toscano et al., 2016). Proline content has a significant and remarkably increase under drought stress conditions. With the intensification of water stress (40% CC), the proline content showed a further increase compared to the plants under moderate (60% CC) and non-stress conditions (100% CC). The application of LME reduced the proline accumulation in severe water stress treatments (40% CC). Nevertheless, the application of LME declines levels of proline in severe drought stress treatment. Thus, as indicated by our results, a positive turgor pressure and water balance may be maintained by LME. Furthermore, the ability of plants to overcome lower water potential is supported by the increase of Pro, because as an osmolyte, it has the particularity of additional water uptake buffering the instantaneous effect of the shortage of water (Manivannan et al., 2007).

MDA is the final product of membrane lipid peroxidation and acts as the damage signal of cell membrane (Toscano et al., 2016). Under the effect of irrigation treatments (60% CC and 40% CC) MDA content increased sharply compared to the control plants (100% CC). Treatment with LME extract significantly reduced MDA level in leaves 40% CC plants when compared to drought stressed plants. *Moringa olifera* extract contains a substantial level of calcium which can inhibiting injurious and leakage of membrane as well as stabilizing membrane structure under adverse drought stress conditions (Hanafy, 2017). During drought stress conditions, NADP will become limited in the acceptance of the electrons and so cause the overproduction of ROS. Similar results were observed in *Amaranthus tricolor* L. (Sarker and Oba, 2018), *Gossypium herbaceum* L. (Deeba et al., 2012), *Adonis amurensis* Regel & Radde and *A. pseudoamurensis* W.T.Wang (Gao et al., 2020). When plants were under stress, the overproduction of ROS and free radicals can cause membrane lipid peroxidation, which leads to cell damage or death (Gao et al., 2020). In particular, this was observed in water stress conditions, with an increase in oxidative damage in plants due to the production and accumulation of reactive oxygen species (ROS) (Toscano et al., 2016). To overcome this condition, different studies have shown that drought-tolerant plants have strong scavenging systems that help them maintain low levels of ROS and prevent membrane lipid peroxidation during stress (Faize et al., 2011). CAT, SOD, POD and APX are important antioxidant enzymes in the plant scavenging system; among them, SOD converts O_2^- into H_2O_2 and O_2, and CAT and GPX scavenge H_2O_2 into H_2O (Reddy et al., 2004). In our study, biostimulant application increased GPX and SOD activities in plants grown under severe water deficit, and this facilitated the ROS scavenging and maintenance of plant growth under stress. Enhancing antioxidant defense systems in response to LME application, therefore, enhanced scavenged ROS, and thus improved membrane stability which in turn increased tolerance to high

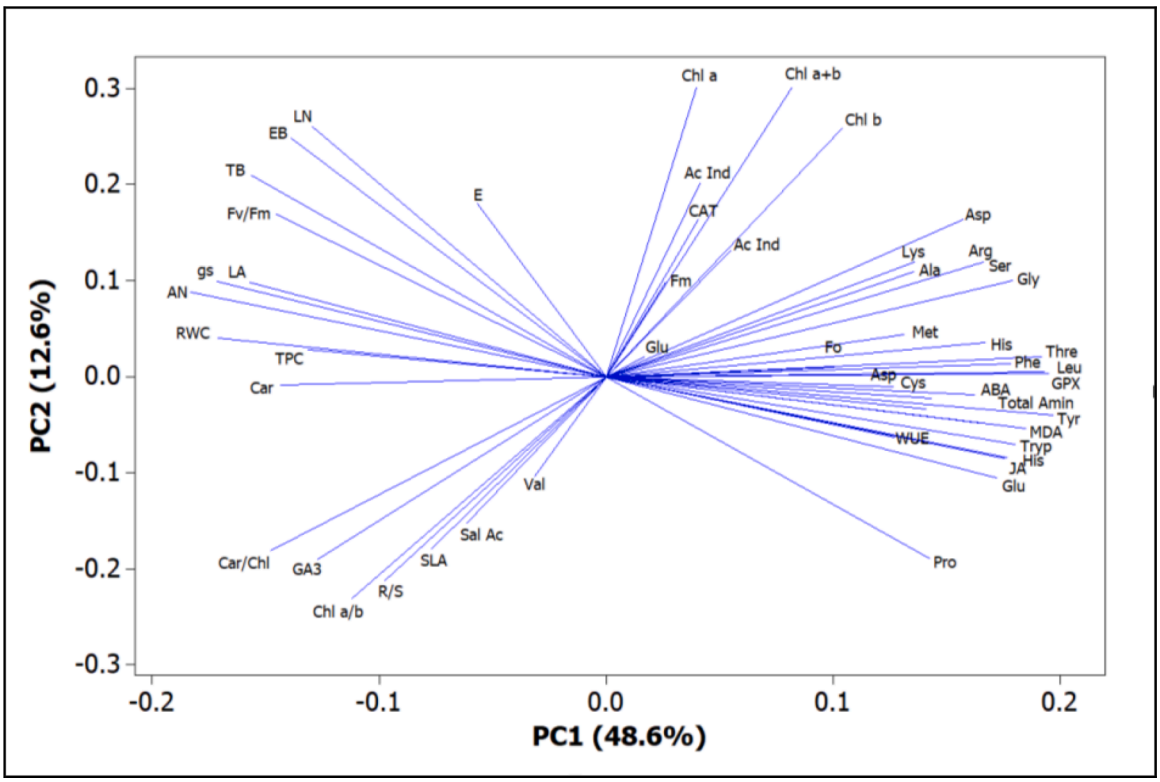

Fig. 8. Principal component analysis (PC1 vs PC2) of petunia plants subjected to water stress treatments: loading plot and distribution of parameters in the consensus space.

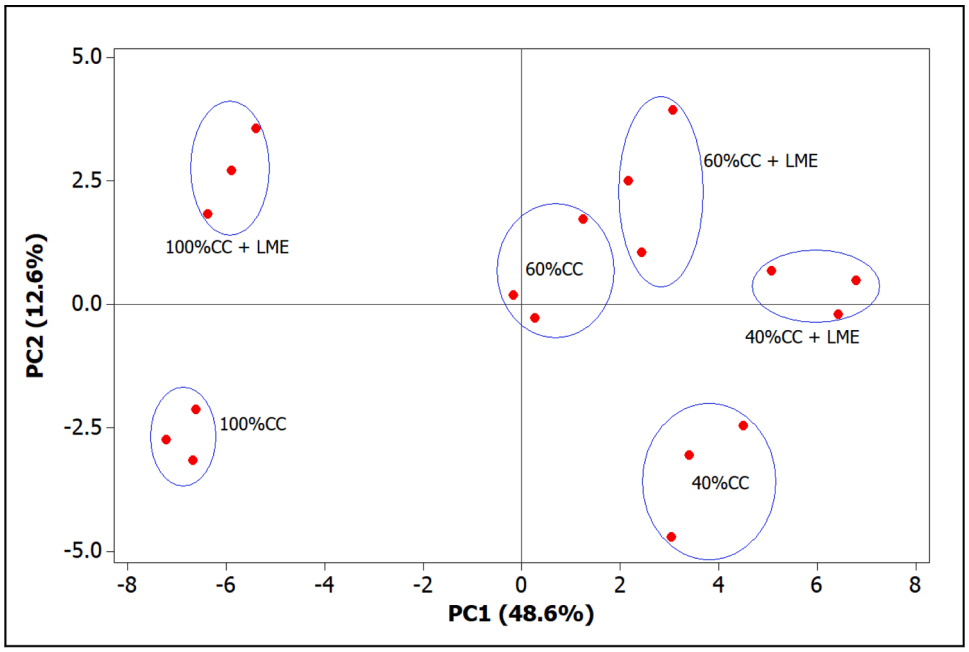

Fig. 9. Principal component analysis (PCA) showing the separation of treatments for the petunia plants by the score plot.

drought stress conditions. Among these indicators, SOD plays a crucial role and it is regarded as the first line of defense scavenging active oxygen free radicals (Lu et al., 2020).

Drought stress also affects the production of endogenous level of hormones, like Abscisic acid (ABA), Jasmonic acid (JA), Ethylene (Eth), Gibberellins (GA), Auxins (Aux), Salicylic acid (SA) and Cytokinins (CK) (Ullah et al., 2018). These phytohormones have an important function in regulating plant growth development and responses to drought stress conditions.

ABA synthesis is one of the fastest responses of plants to drought stress causing stomatal closure. In addition, SA is involved in the regulation of drought responses, enhanced antioxidant enzymatic activities together with other physio-biochemical traits (Liu et al., 2022). In our experiment, endogenous ABA and JA levels in stressed plants increased

compared to control plants. The active derivative of jasmonic acid, also known as jasmonates, has an important role in controlling the response to various biotic and abiotic stresses (Ullah et al., 2018). JA enhance drought tolerance by different procedures, including the closing of stomata, root development and scavenging of ROS.

As a mechanism of adaptation to abiotic stresses, plants synthetize a series of secondary metabolites, in particular the phenolic compounds, and specifically flavonoids (Di Ferdinando et al., 2014).

Different studies presented that plants would accumulate flavonoids and phenols with increasing of abiotic stress and in particular under drought, such as *Chrysanthemum morifolium* Ramat (Hodaei et al., 2018), *Capsicum* species (Okunlola et al., 2017), and *Achillea* species (Gharibi et al., 2016). High levels of these compounds in plants under drought stress mean that the plants adapt to the drought environment (Liu et al., 2011). In accordance with this, the petunia plants had high levels of TPC under moderate and severe water deficit, but not in severe deficit irrigation treated with LME. This explains that the species adapts to water deficit by scavenging ROS by TPC, showing the highest values in biostimulant treatment. Furthermore, results also showed that LME significantly enhanced total phenols content in 100% CC+LME and 60% CC+LME and this may be due to the high phenol content in LME, which might influence the endogenous content of total phenols in petunia plants. This could be also attributed to the minerals, β-carotene, and vitamins content of moringa leaves that could have increased phenol metabolism in plants (Abdalla et al., 2013).

Organic osmolytes, such as soluble sugars, accumulate in plants in response to abiotic stress (Hsu et al., 2003). This was observed in our study, while the accumulation of total soluble sugars in the leaf increased significantly under water stress conditions. Increasing the activity of the sucrose-phosphatase enzyme, which has a crucial role in the metabolism of starch and sucrose, would result in a higher amount of sucrose (Cheikh and Brenner, 1992). The sugar accumulation acts as osmolytes to keep proteins, cell turgor and membrane stability from damage (Kaplan and Guy, 2004).

5. Conclusions

The response of petunia plants to water deficit was related to its ability to decrease aerial growth and to modify leaf gas exchange, increasing secondary osmolytes and enzyme activity to contrast the ROS activity. The plants grown under moderate water deficit are able to maintain a final biomass similar to plants grown under well irrigated conditions. Water stress did not reduce chlorophyll contents but led to decreased chlorophyll a/b. Furthermore, this species has shown to improve its water use efficiency along mild to severe stress, in particular when treated with LME. Leaf moringa application improved drought resistance of the plants, and in particular reduced the water deficit effects modifying the growth parameters, proline and MDA content and enzyme activity. Therefore, the use of petunia plants treated with leaf moringa extract in gardening or urban projects can be an option in areas where environmental conditions are unfavorable and lack of water is the main limiting factor.

CRediT authorship contribution statement

S. Toscano: Conceptualization, Visualization, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **M.J. Gómez-Bellot:** Methodology, Writing – original draft, Writing – review & editing. **D. Romano:** Conceptualization, Visualization, Investigation, Writing – original draft, Writing – review & editing. **M.J. Sánchez-Blanco:** Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abd El-Mageed, T.A., Semida, W.M., Rady, M.M., 2017. Moringa leaf extract as biostimulant improves water use efficiency, physio-biochemical attributes of squash plants under deficit irrigation. Agric. Water Manag. 193, 46–54. https://doi.org/10.1016/j.agwat.2017.08.004.

Abdalla, M.M., 2013. The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. *sativa*) plants. Int. J. Plant Physiol. Biochem. 5, 42–49. https://doi.org/10.5897/IJPPB2012.026.

Aguilera, J., Bischof, K., Karsten, U., Hanelt, D., Wiencke, C., 2002. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. Mar. Biol. 140, 1087–1095. https://doi.org/10.1007/s00227-002-0792-y.

Ahmad, P., John, R., Sarwat, M., Umar, S., 2008. Responses of proline, lipid peroxidation and antioxidative enzymes in two varieties of *Pisum sativum* L. under salt stress. Int. J. Plant. Prod. 2, 353–366.

Ali, M.M., Sani, M.Z.B., Hi, K.K., Yasir, S.M., Critchley, A.T., Hurtado, A.Q., 2018. The comparative efficiency of a brown algal-derived biostimulant extract (AMPEP), with and without supplemented PGRs: the induction of direct, axis shoots as applied to the propagation of vegetative seedlings for the successful mass cultivation of three commercial strains of Kappaphycus in Sabah. Malaysia. J. Appl. Psychol. 30 (3), 1913–1919. https://doi.org/10.1007/s10811-017-1366-1.

Álvarez, S., Bañón, S., Sánchez-Blanco, M.J., 2013. Regulated deficit irrigation in different phenological stages of potted geranium plants: water consumption, water relations and ornamental quality. Acta Physiol. Plant. 35 (4), 1257–1267. https://doi.org/10.1007/s11738-012-1165-x.

Álvarez, S., Sánchez-Blanco, M.J., 2013. Changes in growth rate, root morphology and water use efficiency of potted *Callistemon citrinus* plants in response to different levels of water deficit. Sci. Hortic. 156, 54–62. https://doi.org/10.1016/j.scienta.2013.03.024.

Anjum, S.A., Farooq, M., Wang, L.C., Xue, L.L., Wang, S.G., Wang, L., Chen, M., 2011. Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. Plant Soil Environ. 57 (7), 326–331. https://doi.org/10.17221/41/2011-PSE.

Antonić, D., Milošević, S., Cingel, A., Lojić, M., Trifunović-Momčilov, M., Petrić, M., Subotić, A., Simonović, A., 2016. Effects of exogenous salicylic acid on *Impatiens walleriana* L. grown *in vitro* under polyethylene glycol-imposed drought. S. Afr. J. Bot. 105, 226–233. https://doi.org/10.1016/j.sajb.2016.04.002.

Ashraf, R., Sultana, B., Iqbal, M., Mushtaq, M., 2016. Variation in biochemical and antioxidant attributes of Raphanus sativus in response to foliar application of plant leaf extracts as plant growth regulator. J. Genet. Eng. Biotechnol 14, 1–8. https://doi.org/10.1016/j.jgeb.2016.08.003.

Bayona-Morcillo, P.J., Plaza, B.M., Gómez-Serrano, C., Rojas, E., Jimenez-Becker, S., 2020. Effect of the foliar application of Cyanobacterial hydrolysate (*Arthrospira platensis*) on the growth of *Petunia hybrida* under salinity conditions. J. Appl. Phycol. 32, 4003–4011. https://doi.org/10.1007/s10811-020-02192-3.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Bray, E.A., 2004. Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*. J. Exp. Bot. 55 (407), 2331–2341. https://doi.org/10.1093/jxb/erh270.

Bulgari, R., Cocetta, G., Trivellini, A., Vernieri, P., Ferrante, A., 2015. Biostimulants and crop responses: a review. Biol. Agric. Hortic. 31 (1), 1–17. https://doi.org/10.1080/01448765.2014.964649.

Bulgari, R., Franzoni, G., Ferrante, A., 2019. Biostimulants application in horticultural crops under abiotic stress conditions. Agronomy 9, 306. https://doi.org/10.3390/agronomy9060306.

Cameron, R.W.F., Harrison-Murray, R.S., Atkinson, C.J., Judd, H.L., 2006. Regulated deficit irrigation—A means to control growth in woody ornamentals. J. Hortic. Sci. Biotechnol. 81, 435–443. https://doi.org/10.1080/14620316.2006.11512085.

Chaturvedi, A.K., Surendran, U., Gopinath, G., Chandran, K.M., Anjali, N.K., Ct, M.F., 2019. Elucidation of stage specific physiological sensitivity of okra to drought stress through leaf gas exchange, spectral indices, growth and yield parameters. Agric. Water Manag. 222, 92–104. https://doi.org/10.1016/j.agwat.2019.05.041.

Cheikh, N., Brenner, M.L., 1992. Regulation of key enzymes of sucrose biosynthesis in soybean leaves: effect of dark and light conditions and role of gibberellins and abscisic acid. Plant Physiol 100 (3), 1230–1237. https://doi.org/10.1104/pp.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought—From genes to the whole plant. Funct. Plant Biol. 30 (3), 239–264. https://doi.org/10.1071/FP02076.

Chyliński, W.K., Łukaszewska, A.J., Kutnik, K., 2007. Drought response of two bedding plants. Acta Physiol. Plant. 29 (5), 399–406. https://doi.org/10.1007/s11738-007-0073-y.

da Silva, T.I., Dias, M.G., de Araújo, N.O., de Sousa Santos, M.N., Ribeiro, W.S., dos Santos Filho, F.B., Dias, T.J., Barbosa, J.G., Grossi, J.A.S., 2022. Spermine reduces the harmful effects of drought stress in *Tropaeolum majus*. Sci. Hortic. 304, 111339 https://doi.org/10.1016/j.scienta.2022.11133.

Deeba, F., Pandey, A.K., Ranjan, S., Mishra, A., Singh, R., Sharma, Y.K., Shirke, P.A., Pandey, V., 2012. Physiological and proteomic responses of cotton (*Gossypium herbaceum* L.) to drought stress. Plant Physiol. Biochem. 53, 6–18. https://doi.org/10.1016/j.plaphy.2012.01.002.

Desoky, E.S.M., Merwad, A.R.M., Rady, M.M., 2018. Natural biostimulants improve saline soil characteristics and salt stressed-sorghum performance. Commun Soil Sci Plant Anal 49 (8), 967–983. https://doi.org/10.1080/00103624.2018.1448861.

Di Ferdinando, M., Brunetti, C., Agati, G., Tattini, M., 2014. Multiple functions of polyphenols in plants inhabiting unfavorable Mediterranean areas. Environ. Exp. Bot. 103, 107–116. https://doi.org/10.1016/j.envexpbot.2013.09.012.

Din, J., Khan, S.U., Ali, I., Gurmani, A.R., 2011. Physiological and agronomic response of canola varieties to drought stress. J. Anim. Plant Sci. 21 (1), 78–82.

Elzaawely, A.A., Ahmed, M.E., Maswada, H.F., Xuan, T.D., 2016. Enhancing growth, yield, biochemical, and hormonal contents of snap bean (*Phaseolus vulgaris* L.) sprayed with moringa leaf extract. Arch. Agron. Soil Sci. 1–13. https://doi.org/10.1080/03650340.2016.1234042.

Fahad, S., Bajwa, A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M.Z., Alharby, H., Wu, C., Wang, D., Huang, J., 2017. Crop production under drought and heat stress: plant responses and management options. Front. Plant Sci. 8, 1147. https://doi.org/10.3389/fpls.2017.01147.

Faize, M., Burgos, L., Faize, L., Piqueras, A., Nicolas, E., Barba-Espin, G., Hernandez, J.A., 2011. Involvement of cytosolic ascorbate peroxidase and Cu/Zn-superoxide dismutase for improved tolerance against drought stress. J. Exp. Bot. 62 (8), 2599–2613. https://doi.org/10.1093/jxb/erq432.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., Lichtfouse, E., Navarrete, M., Debaeke, P., Véronique, S., Alberola, C., 2009. Plant drought stress: effects, echmanisms and management. Sustainable Agriculture. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-2666-8_12.

Fu, J., Huang, B., 2001. Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environ. Exp. Bot. 45 (2), 105–114. https://doi.org/10.1016/S0098-8472(00)00084-8.

Gao, S., Wang, Y., Yu, S., Huang, Y., Liu, H., Chen, W., He, X., 2020. Effects of drought stress on growth, physiology and secondary metabolites of two *Adonis* species in Northeast China. Sci. Hortic. 259, 108795 https://doi.org/10.1016/j.scienta.2019.108795.

Gharibi, S., Tabatabaei, B.E.S., Saeidi, G., Goli, S.A.H., 2016. Effect of drought stress on total phenolic, lipid peroxidation, and antioxidant activity of *Achillea* species. Appl. Biochem. Biotechnol. 178, 796–809. https://doi.org/10.1007/s12010-015-1909-3, 2016.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide occurrence in higher plants. Plant Physiol. 59, 309–314. https://doi.org/10.1104/pp.59.2.309.

Godlewska, K., Biesiada, A., Michalak, I., Pacyga, P., 2019. The effect of plant-derived biostimulants on white head cabbage seedlings grown under controlled conditions. Sustainability 11, 5317. https://doi.org/10.3390/su11195317.

Gómez-Bellot, M.J., Lorente, B., Ortuño, M.F., Medina, S., Gil-Izquierdo, Á., Bañón, S., Sánchez-Blanco, M.J., 2021. Recycled wastewater and reverse osmosis brine use for halophytes irrigation: differences in physiological, nutritional and hormonal responses of *Crithmum maritimum* and *Atriplex halimus* plants. Agronomy 11 (4), 627. https://doi.org/10.3390/agronomy11040627.

Guo, R., Shi, L., Jiao, Y., Li, M., Zhong, X., Gu, F., Liu, Q., Xia, X., Li, H., 2018. Metabolic responses to drought stress in the tissues of drought-tolerant and drought-sensitive wheat genotype seedlings. AOB Plants 10 (2). https://doi.org/10.1093/aobpla/ply016 ply016.

Hanafy, R.S., 2017. Using *Moringa olifera* leaf extract as a bio-fertilizer for drought stress mitigation of *Glycine* max L. plants. Egypt. J. Bot. 57 (2), 281–292. https://doi.org/10.21608/ejbo.2017.596.1027.

Hessini, K., Ghandour, M., Albouchi, A., Soltani, A., Werner, K.H., Abdelly, C., 2008. Biomass production, photosynthesis, and leaf water relations of *Spartina alterniflora* under moderate water stress. J. Plant Res. 121 (3), 311–318. https://doi.org/10.1007/s10265-008-0151-2.

Hodaei, M., Rahimmalek, M., Arzani, A., Talebi, M., 2018. The effect of water stress on phytochemical accumulation, bioactive compounds and expression of key genes involved in flavonoid biosynthesis in *Chrysanthemum morifolium* L. Ind. Crop. Prod. 120, 295–304. https://doi.org/10.1016/j.indcrop.2018.04.073.

Howladar, S.M., 2014. A novel *Moringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. Ecotoxicol Environ Saf 100, 69–75. https://doi.org/10.1016/j.ecoenv.2013.11.022.

Hsu, S.Y., Hsu, Y.T., Kao, C.H., 2003. The effect of polyethylene glycol on proline accumulation in rice leaves. Biol. Plant. 46 (1), 73–78. https://doi.org/10.1023/A:1022362117395.

Jaramillo, R.E., Nord, E.A., Chimungu, J.G., Brown, K.M., Lynch, J.P., 2013. Root cortical burden influences drought tolerance in maize. Ann. Bot. 112 (2), 429–437. https://doi.org/10.1093/aob/mct069.

Kaplan, F., Guy, C.L., 2004. β-Amylase induction and the protective role of maltose during temperature shock. Plant Physiol. 135 (3), 1674–1684. https://doi.org/10.1104/pp.104.040808.

Kim, J., Malladi, A., Van Iersel, M.W., 2012. Physiological and molecular responses to drought in Petunia: the importance of stress severity. J. Exp. Bot. 63 (18), 6335–6345. https://doi.org/10.1093/jxb/ers285.

Kıvrak, İ., Kıvrak, Ş., Harmandar, M., 2014. Free amino acid profiling in the giant puffball mushroom (*Calvatia gigantea*) using UPLC–MS/MS. Food Chem 158, 88–92. https://doi.org/10.1016/j.foodchem.2014.02.108.

Kulcheski, F.R., Muschner, V.C., Lorenz-Lemke, A.P., Stehmann, J.R., Bonatto, S.L., Salzano, F.M., Freitas, L.B., 2006. Molecular phylogenetic analysis of *Petunia* Juss. (Solanaceae). Genetica 126 (1), 3–14. https://doi.org/10.1007/s10709-005-1427-2.

Kunicki, E., Grabowska, A., Sękara, A., Wojciechowska, R., 2010. The effect of cultivar type, time of cultivation, and biostimulant treatment on the yield of spinach (*Spinacia oleracea* L.). Folia Hortic. 22 (2), 9–13. https://doi.org/10.2478/fhort-2013-0153.

Li, G., Wan, S., Zhou, J., Yang, Z., Qin, P., 2010. Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. Ind. Crop. Prod. 31, 13–19. https://doi.org/10.1016/j.indcrop.2009.07.015.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Method Enzymol. 148, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

Lin, B., Raza, M.Y., 2019. Analysis of energy related CO_2 emissions in Pakistan. J. Clean. Prod. 219, 981–993. https://doi.org/10.1016/j.jclepro.2019.02.112.

Lin, Y., Jones, M.L., 2022. Evaluating the growth-promoting effects of microbial biostimulants on greenhouse floriculture crops. HortSci 57, 97–109. https://doi.org/10.21273/HORTSCI16149-21.

Liu, J., Qiu, G., Liu, C., Li, H., Chen, X., Fu, Q., Lin, Y., Guo, B., 2022. Salicylic acid, a multifaceted hormone, combats abiotic stresses in plants. Life 12 (6), 886. https://doi.org/10.3390/life12060886.

Liu, H., Wang, X., Wang, D., Zou, Z., Liang, Z., 2011. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza*. Bunge. Ind. Crop. Prod. 33, 84–88. https://doi.org/10.1016/j.indcrop.2010.09.006.

Lobell, D.B., Gourdji, S.M., 2012. The influence of climate change on global crop productivity. Plant Physiol 160 (4), 1686–1697. https://doi.org/10.1104/pp.112.208298.

Lu, K., Cheng, Y., Li, W., Li, Y., Zeng, R., Song, Y., 2020. Activation of CncC pathway by ROS burst regulates cytochrome P450 CYP6AB12 responsible for λ-cyhalothrin tolerance in *Spodoptera litura*. J. Hazard. Mater. 387, 121698 https://doi.org/10.1016/j.jhazmat.2019.121698.

Manivannan, P., Jaleel, C.A., Sankar, B., Kishorekumar, A., Somasundaram, R., Lakshmanan, G.A., Panneerselvam, R., 2007. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. Colloids Surf. B Biointerfaces 59 (2), 141–149. https://doi.org/10.1016/j.colsurfb.2007.05.002.

Müller, M., Munné-Bosch, S., 2011. Rapid and sensitive hormonal profiling of complex plant samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Plant Methods 7 (1), 1–11. https://doi.org/10.1186/1746-4811-7-37.

Nasir, M., Sattar Khan, A., Basra, S.M.A., Malik, A.U., 2016. Foliar applications of moringa leaf extract, potassium and zinc influence yield and fruit quality of 'Kinnow' mandarin. Sci. Hortic. 210, 227–235. https://doi.org/10.1016/j.scienta.2016.07.032.

Nimbalkar, M.S., Pai, S.R., Pawar, N.V., Oulkar, D., Dixit, G.B., 2012. Free amino acid profiling in grain Amaranth using LC–MS/MS. Food Chem. 134 (4), 2565–2569. https://doi.org/10.1016/j.foodchem.2012.04.057.

Niu, G., Rodriguez, D.S., 2009. Growth and physiological responses of four rose rootstocks to drought stress. J. Am. Soc. Hortic. 134 (2), 202–209. https://doi.org/10.21273/JASHS.134.2.202.

Okunlola, G.O., Olatunji, O.A., Akinwale, R.O., Tariq, A., Adelusi, A.A., 2017. Physiological response of the three most cultivated pepper species (*Capsicum* spp.) in Africa to drought stress imposed at three stages of growth and development. Sci. Hortic. 224, 198–205. https://doi.org/10.1016/j.scienta.2017.06.020.

Oraee, A., Tehranifar, A., 2020. Evaluating the potential drought tolerance of pansy through its physiological and biochemical responses to drought and recovery periods. Sci. Hortic. 265, 109225 https://doi.org/10.1016/j.scienta.2020.109225.

Pál, M., Tajti, J., Szalai, G., Peeva, V., Végh, B., Janda, T., 2018. Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. Sci. Rep. 8 (1), 1–12. https://doi.org/10.1038/s41598-018-31297-6.

Rady, M.M., Bhavya, V.C., Howladar, S.M., 2013. Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. Sci. Hortic. 162, 63–70. https://doi.org/10.1016/j.scienta.2013.07.046.

Rafi, Z.N., Kazemi, F., Tehranifar, A., 2019. Effects of various irrigation regimes on water use efficiency and visual quality of some ornamental herbaceous plants in the field. Agric. Water Manag. 212, 78–87. https://doi.org/10.1016/j.agwat.2018.08.012Get rights and content.

Reddy, V.N., Kasahara, E., Hiraoka, M., Lin, L.R., Ho, Y.S., 2004. Effects of variation in superoxide dismutases (SOD) on oxidative stress and apoptosis in lens epithelium. Exp. Eye Res. 79 (6), 859–868. https://doi.org/10.1016/j.exer.2004.04.005.

Rehman, H., Iqbal, H., Basra, S., Afzal, I., Farooq, M., Wakeel, A., Ning, W., 2015. Seed priming improves early seedling vigor, growth and productivity of spring maize. J. Integr. Agric. 14, 1745–1754. https://doi.org/10.1016/S2095-3119(14)61000-5.

Romano, D., La Fornara, G., Tribulato, A., Toscano, S., 2022. Can Moringa leaf spray treatment increase the nutraceutical properties of radish baby leaf? Horticulturae 8 (8), 671. https://doi.org/10.3390/horticulturae8080671.

Rosales-Serna, R., Kohashi-Shibata, J., Acosta-Gallegos, J.A., Trejo-López, C., Ortiz-Cereceres, J., Kelly, J.D., 2004. Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. Field Crops Res. 85 (2–3), 203–211. https://doi.org/10.1016/S0378-4290(03)00161-8.

Ruley, A.T., Sharma, N.C., Sahi, S.V., 2004. Antioxidant defense in a lead accumulating plant, *Sesbania drummondii*. Plant Physiol. Biochem. 42, 899–906. https://doi.org/10.1016/j.plaphy.2004.12.001.

Saini, I., Aggarwal, A., Kaushik, P., 2019. Influence of biostimulants on important traits of *Zinnia elegans* Jacq. under open field conditions. Int. J. Agron. 2019, e3082967 https://doi.org/10.1155/2019/3082967.

Sánchez-Blanco, M.J., Álvarez, S., Navarro, A., Bañón, S., 2009. Changes in leaf water relations, gas exchange, growth and flowering quality in potted geranium plants irrigated with different water regimes. J. Plant Physiol. 166 (5), 467–476. https://doi.org/10.1016/j.jplph.2008.06.015.

Sánchez-Blanco, M.J., Ortuño, M.F., Bañon, S., Álvarez, S., 2019. Deficit irrigation as a strategy to control growth in ornamental plants and enhance their ability to adapt to drought conditions. J. Hortic. Sci. Biotechnol. 94 (2), 137–150. https://doi.org/10.1080/14620316.2019.1570353.

Sarker, U., Oba, S., 2018. Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in *Amaranthus tricolor*. Appl. Biochem. Biotech. 186 (4), 999–1016. https://doi.org/10.1007/s12010-018-2784-5.

Toscano, S., Farieri, E., Ferrante, A., Romano, D., 2016. Physiological and biochemical responses in two ornamental shrubs to drought stress. Front. Plant. Sci. 7, 645. https://doi.org/10.3389/fpls.2016.00645.

Toscano, S., Ferrante, A., Branca, F., Romano, D., 2021. Enhancing the quality of two species of baby leaves sprayed with Moringa Leaf Extract as biostimulant. Agronomy 11, 1399. https://doi.org/10.3390/agronomy11071399.

Tribulato, A., Toscano, S., Di Lorenzo, V., Romano, D., 2019. Effects of water stress on gas exchange, water relations and leaf structure in two ornamental shrubs in the Mediterranean area. Agronomy 9 (7), 381. https://doi.org/10.3390/agronomy9070381.

Trivedi, K., Vijay Anand, K.G., Kubavat, D., Patidar, R., Ghosh, A., 2018. Drought alleviatory potential of *Kappaphycus* seaweed extract and the role of the quaternary ammonium compounds as its constituents towards imparting drought tolerance in *Zea mays* L. J. Appl. Phycol. 30 (3), 2001–2015. https://doi.org/10.1007/s10811-017-1375-0.

Ullah, A., Sun, H., Yang, X., Zhang, X., 2018. A novel cotton WRKY gene, GhWRKY6-like, improves salt tolerance by activating the ABA signaling pathway and scavenging of reactive oxygen species. Physiol Plant 162, 439–454. https://doi.org/10.1111/ppl.12651.

Valdecantos, A., Cortina, J., Vallejo, V.R., 2011. Differential field response of two Mediterranean tree species to inputs of sewage sludge at the seedling stage. Ecol. Eng. 37 (9), 1350–1359. https://doi.org/10.1016/j.ecoleng.2011.03.017.

Watkinson, J.I., Sioson, A.A., Vasquez-Robinet, C., Shukla, M., Kumar, D., Ellis, M., Grene, R., 2003. Photosynthetic acclimation is reflected in specific patterns of gene expression in drought-stressed loblolly pine. Plant Physiol. 133 (4), 1702–1716. https://doi.org/10.1104/pp.103.026914.

WWAP (World Water Assessment Programme), 2014. The United Nations World Water Development Report 2014: Water and Energy, 2014. UNESCO, Paris, France.

Yadollahi, A., Arzani, K., Ebadi, A., Wirthensohn, M., Karimi, S., 2011. The response of different almond genotypes to moderate and severe water stress in order to screen for drought tolerance. Sci. Hortic. 129 (3), 403–413. https://doi.org/10.1016/j.scienta.2011.04.007.

Yasmeen, A., Basra, S.M.A., Farooq, M., Hussain, N., 2013a. Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. Plant Growth Regul. 69, 225–233. https://doi.org/10.1007/s10725-012-9764-5.

Yasmeen, A., Basra, S.M.A., Wahid, A., Nouman, W., Rehman, H.U.R., 2013b. Exploring the potential of Moringa oleifera leaf extract (MLE) as a seed priming agent in improving wheat performance. Turk. J. Bot. 37, 512–520. https://doi.org/10.3906/bot-1205-19.

Yemm, E.W., Willis, A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508–514. https://doi.org/10.1042/bj0570508.

Zeljković, S., Parađiković, N., Tkalec Kojić, M., Mladenović, E., 2021. Effect of biostimulant application on development of pansy (*Viola tricolor* var. *hortensis* DC.) seedlings. J. Cent. Eur. Agric. 22 (3), 596–601. https://doi.org/10.5513/JCEA01/22.3.3191.

Zulfiqar, F., Casadesús, A., Brockman, H., Munné-Bosch, S., 2020. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. Plant Sci. 295, 110194 https://doi.org/10.1016/j.plantsci.2019.110194.