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Comparative analysis of LED priming effects on two medicinal lemon balm genotypes one and three weeks post-drought stress

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Abstract

Background Light is essential for producing high-quality plants. The advancement of light-emitting diode technology has unlocked new opportunities for growing plants in controlled settings. In this study, the effects of light-emitting diodes priming and drought stress on some physiological and biochemical parameters were studied in two *Melissa officinalis* genotypes (Ilam and Isfahan) one and three weeks after drought stress. The experiments were conducted in a factorial arrangement within a completely randomized design with three replications.

Results Drought stress reduced growth indicators such as fresh and dry weights of aerial parts, leaf number, and relative water content. Light-emitting diode priming relieved such reductions in both genotypes. The accumulation of phenolic compounds, anthocyanin, and levels of proline, along with the activity of the enzyme phenylalanine ammonia-lyase, increased under drought stress, with the maximum increase achieved under red + blue and blue light-emitting diode light-primed plants. Especially in the Ilam genotype, phenylalanine ammonia-lyase enzyme activities and the accumulation of phenolic compounds were remarkably enhanced by the use of red + blue light-emitting diode light. Also, abscisic acid showed higher values under drought stress and the highest in pre-treatments with red + blue and red light-emitting diodes.

Conclusion The effects of different treatments on the physiological indices showed that drought tolerance in *Melissa officinalis* was improved due to the priming of red + blue light-emitting diode in both genotypes. Thus, our results emphasized the use of light-emitting diode priming as a useful method to enhance the drought resistance of medicinal plants.

Keywords Light priming, Drought stress, Medicinal plant, Drought-resistant genotypes, Red + blue LED, Priming one and three weeks after drought

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Introduction

Drought stress is one of the complex stresses that mediate physiological, morphological, biochemical, and molecular changes in plants, causing serious damage to growth and development. It has been found to adversely affect the quantity and quality of growth and yield of plants. Plants usually adopt mechanisms for drought tolerance through the maintenance of cellular water homeostasis and enhanced production of hormones and secondary metabolites to mitigate reactive oxygen species (ROS) generated under stress conditions [1, 2]. Plant hormones modulate plant responses to stress factors, like drought. Among these plant hormones, the role of abscisic acid (ABA) is critical. The synthesis of ABA in the roots during drought is transported to the aerial part, particularly the leaves [3]. The ABA induces signaling into guard cells through modifications in ion transport, promoting stomata closure, and reducing transpiration, and photosynthesis. The levels of ABA rise in response to drought stress, but this is often specific to the species or the variety of a particular plant concerning its level of drought tolerance [4, 5].

Another important mechanism for drought tolerance is the accumulation of compatible solutes for osmotic adjustment. These low molecular weight compounds can accumulate at high concentrations without interfering with cellular metabolism [6]. They enhance osmotic pressure, water uptake, and cell structural stability. Proline is one of the most abundant compatible solutes that accumulate in the case of drought stress as a result of enhanced synthesis and reduced degradation. Proline maintains osmotic potential, protects cells against oxidative damage, and balances energy between chloroplasts and mitochondria under drought stress [7].

Water stress also diverts carbon allocation from growth to synthesizing secondary metabolites. This metabolic shift enables plants to produce compounds used in defense mechanisms, thus allowing them to cope with stress [8]. For example, *Hypericum brasiliense* plants under drought stress accumulated higher concentrations and total amounts of phenolic compounds than controls [9]. Similar trends were observed in *Salvia miltiorrhiza*, where the concentration of phenolic compounds increased despite the reduced total content under drought stress [10]. Lemon balm also showed increased monoterpene concentrations under drought conditions [11]. Other approaches to enhance drought tolerance involve genetic strategies. However, because of the high cost and requirements for technical expertise, in addition to the generally low percentage of success, obtaining genetically modified plants resistant to drought is not yet widely possible [1]. Stress priming has been considered an alternative approach to enhance stress tolerance. Stress priming is a phenomenon whereby the mild initial stress applied to plants induces faster and more efficient responses to subsequent stresses [12, 13]. It is associated with stress memory, which involves physiological changes and transcriptional, proteomic, and epigenetic factors throughout the life cycle of a plant [1].

Light priming is a technique in which the seeds or seedlings are briefly illuminated. This triggers physiological changes within the plant, such as hormone production and gene activation, which leads to better growth and stress tolerance [14]. Poudel et al. found that red LED light significantly increased the levels of the plant hormone ABA in grape skins compared to blue LED or no LED treatment. This increase in ABA was associated with higher expression of the *VvNCED1* gene (involved in ABA synthesis) and *VvCYP707A1* gene (involved in ABA degradation) in grapes exposed to red LED [15]. Huang et al. [16] suggested that supplementation with blue and yellow LED lights could be a promising strategy for improving the drought tolerance of faba bean plants by reducing leaf size, increasing the net photosynthetic rate, increasing stomatal conductance, and strengthening the defense system. Under water stress, seedlings of *Eucalyptus benthamii* grown under blue light LEDs exhibited improved performance, characterized by a slower decline in photosynthesis, maintained water use efficiency, and stable transpiration rates [17].

Lemon balm, scientifically designated *Melissa officinalis* L., is a medicinal plant belonging to the Lamiaceae family, which exhibits various therapeutic properties attributed to its essential oil content and the presence of phenolic acids [18]. This study employed light-emitting diode (LED) light priming as an initial stressor to enhance the physiological and biochemical attributes of lemon balm plants one and three weeks post-drought stress. The objective of utilizing this pretreatment with LED lights was to augment stress memory and enhance drought tolerance in lemon balm plants. The investigation aims to determine the efficacy of this stress memory after one week and three weeks following drought stress.

Materials and methods

Plant materials and treatments

This experiment was conducted from 2013 to 2015 in the greenhouse of Shahrekord University and the Seed and Plant Breeding Laboratory of the Isfahan University of Technology. The experiment had a completely randomized design. Lemon balm plants were collected from farms in Isfahan and Ilam. Three plants with aerial shoots were transplanted into 12 cm plastic pots (60 in total) filled with loamy-sandy soil at a 1:3 ratio. After one month of uniform seedling growth for the two varieties in the greenhouse, the seedling transplants were transferred into a growth chamber (Arovin Tajhiz Sepadan, Isfahan) located in the Seed and Plant Improvement Laboratory

of the Isfahan University of Technology. The incubator was supplemented with LED lamps that emitted red (650 nm), blue (460 nm), red + blue at a ratio of 30:70, and white (380–760 nm) light spectra with an intensity of 300 μmol m^{-2} s^{-1}, while maintaining a 16:8 h photoperiod and a temperature of 25 ± 2 °C for a month. Twelve pots per genotype, six for Ilam and six for Isfahan genotypes were kept in greenhouse conditions (25 ± 2 °C, 16 h light/8 h dark photoperiod) as controls under conditions identical to those of the incubator. When the seedlings from the incubator grew sufficiently, they were transferred to a greenhouse. Soil moisture content was measured using the method described by Kadkhodaie et al. [19]. All pots were fully watered and, after two hours when water drainage ceased, the relative soil moisture content was measured using a ThetaProbe (AT Delta-T Devices SM300, Cambridge, England). Plants were allowed to dry to 30% moisture content, at which time plants from each light pre-treatment were watered. During this period, half of the plants were well-watered, whereas half were under drought stress with watering only when the soil moisture reached 10%. Sampling was conducted one and three weeks after the induction of drought.

Morphometric trait measurements

The plant samples were washed with distilled water and excess moisture was removed using filter paper. The fresh weight of the aerial parts was measured in grams after separating the roots from the collar. The dry weight was measured after keeping the samples in a hot air oven at 65 °C for 48 h. The number of leaves per pot was determined.

Relative water content (RWC) measurement

The RWC was measured according to Turner's method [20]. Fresh leaves were weighed immediately after sampling (FW), soaked in distilled water for 6 h, and reweighed (TW). They were then oven-dried at 70 °C for 24 h, and their dry weight (DW) was recorded. RWC was calculated using the following formula:

$$\mathrm{RWC} = ((\mathrm{FW} - \mathrm{DW}) / (\mathrm{TW} - \mathrm{DW})) \times 100$$

Total phenolic compounds measurement

The total phenolic compounds in the leaves were determined using the method described by Singleton and Rossi [21]. Fresh leaf tissue (0.1 g) was ground in a cold mortar with 3 ml of 80% methanol and centrifuged at 15,000 rpm for 15 min. The supernatant obtained was used for analysis. The reaction mixture contained 30 μL of the extract, 120 μL of Na_2CO_3, and 150 μL of Folin-Ciocalteu reagent. The absorbance was measured at 765 nm after 30 min of incubation in the dark. The phenolic content was calculated using a gallic acid standard curve (25–100 ppm).

Assay of the activity of phenylalanine Ammonia lyase (PAL) enzyme

PAL activity was measured according to the procedure described by Morrison et al. [22]. Fresh sample (0.1 g) tissue was homogenized on ice with Tris-HCl buffer (0.1 M, pH 7.6), containing 20 mM 2-mercaptoethanol and 0.5% PVP. The extract was then left to maintain at 4 °C overnight, after which it was centrifuged at 12,000 × g for 40 min in a refrigerator. Two point five mL of phenylalanine was mixed with 500 μL of the extract containing 12 mM in Tris-HCl buffer (pH 8.5) to determine enzymatic activity.

Instead of phenylalanine, a control was prepared using a buffer. The changes in absorbance at 290 nm were recorded immediately and after 60 min at 30 °C using a JENWAY 6300 spectrophotometer. Activity was expressed as micromoles of cinnamic acid per gram of fresh tissue per min.

Measurement of anthocyanin content

The anthocyanin content was determined spectrophotometrically [23]. Homogenized ground leaves were mixed with 0.1 N HCl, extracted for 3 h in the dark at room temperature, and centrifuged for 5 min at 10,000 × g. The absorbance of the supernatant was read at 511 nm, and anthocyanin content was calculated using the extinction coefficient of cyanidin-3-glucoside (31,760 μM/cm).

Proline content measurement

The proline content was determined according to the method of Troll and Lindsley [24]. The samples were extracted with 90% ethanol and centrifuged at 14,000 × g for 5 min. To 25 μL, 4.75 mL of 70% ethanol and 1 mL of a reaction buffer containing acetic acid, ethanol, ninhydrin, and a water mixture were added. After centrifugation, the mixture was immediately boiled, and the absorbance was measured at 520 nm using a Shimadzu UV 1601 spectrophotometer. The proline content was calculated in μmol/g FW using a standard curve.

ABA extraction and measurement

ABA was extracted using the method described by Kelen et al. [25]. The leaves (2 g) were ground with 10 mL of a solution containing butylated hydroxytoluene and ascorbic acid in 90% methanol, stirred overnight, and filtered. After pH adjustment, the aqueous phase was partitioned against ethyl acetate, dried, and reconstituted in high-performance liquid chromatography (HPLC)-grade methanol for analysis. Chromatographic analysis was performed using a Waters HPLC system (Waters, USA). The system was equipped with a Symmetry-C18 column

Table 1 Analysis of variance (mean squares) of drought, genotype, light, and their interactions on shoot fresh weight, shoot dry weight, and leaf number

Variable	Df	Shoot Fresh Weight	Shoot Dry Weight	Leaf number
Drought	1	2170**	8.3**	34,704*
Genotype	1	33.93**	0.34ns	14,883*
Light	4	209**	9.7**	7078**
Genotype× Drought	4	0.92ns	5.6**	5023**
Light× Drought	4	178**	4.4**	1137**
Genotype × Light	4	29.82**	4.4**	1054**
Genotype × Light × Drought	4	12.86**	1.8**	2562**
Error	40	0.055	0.019	54.11

*and** - significant difference at $P \leq 0.05$ and $P \leq 0.01$, ns- ns, non-significant

(250 × 4.6 mm) and a UV detector (Waters 2487). The mobile phase consisted of a mixture of 0.2% acetic acid and 100% methanol in a 50:50 v/v ratio, with a solvent flow rate of 0.7 mL/min through the column. Measurement and recordings were performed at 265 nm.

Statistical analysis

This experiment used a completely randomized factorial design with three replicates. These factors included two genotypes, Ilam and Isfahan, with five light levels: red, white, blue, red + blue LED, and greenhouse light, along with two levels of drought application: drought and no drought. Data analysis was performed using SAS 8.0 and MSTATC; a comparison of means was made by the LSD test at $P < 0.05$ and $P < 0.01$.

Results

Impact of LED priming on morphological traits at one and three weeks following drought stress

The variance analysis of fresh and dry weights of aerial parts and leaf numbers three weeks after drought stress is presented in Table 1. The effects of drought stress, genotype, and light on the fresh weight of stems and leaf number per pot were statistically significant at the 1% level. While drought stress and light significantly influenced the dry weight of stems, the genotype had no significant effect on this parameter.

Figure 1 shows how different LED light treatments (White, Red + Blue, Red, Blue, and Greenhouse LEDs) affect plant growth (measured by shoot fresh weight, shoot dry weight, and leaf number) under drought and control conditions for two genotypes of Ilam and Isfahan. Under drought stress, all light treatments led to significantly lower values across all three growth metrics compared with the control. However, red + blue LED and red LED were the most effective in maintaining growth under drought conditions for both genotypes. In contrast, greenhouse light resulted in notably lower fresh and dry weights than the other treatments. Both genotypes showed significant declines in fresh weight, dry weight, and leaf number under drought stress, although Ilam generally had slightly better growth outcomes than Isfahan under both drought and control conditions (Fig. 1a-c).

Impact of LED priming on RWC at one and three weeks following drought stress

Over time, drought conditions caused a more noticeable drop in RWC for both Ilam and Isfahan, with the steepest decline observed at the 3-week mark. For instance, in drought-stressed plants, RWC in the Ilam genotype during the first week was twice as high as that in the third week, while in the Isfahan genotype, it was 1.4 times higher. Ilam showed the lowest RWC values after three weeks, particularly under red and blue LED lights, highlighting its greater sensitivity to prolonged drought stress. In contrast, under control conditions, RWC remained relatively high and stable for both genotypes

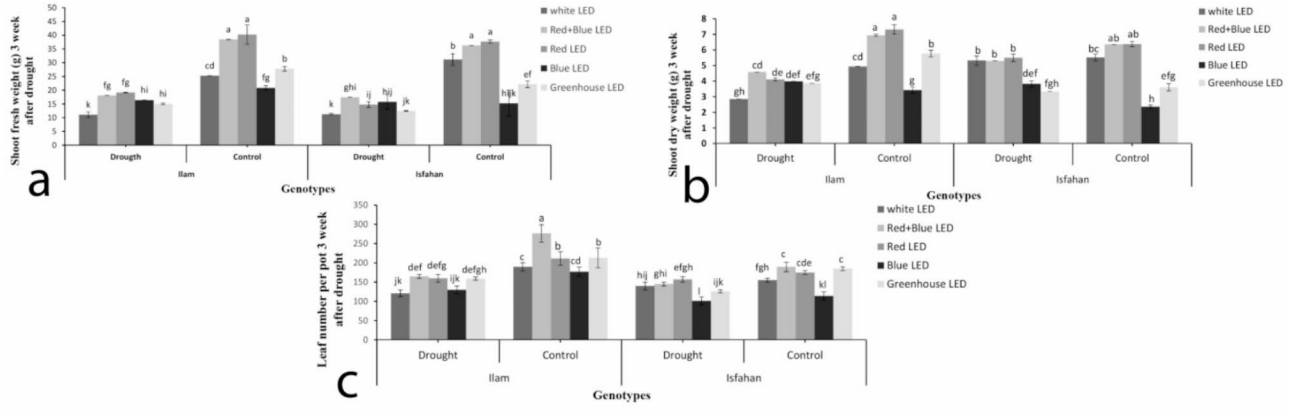

Fig. 1 The impact of LED light priming on shoot fresh weight (**a**), shoot dry weight (**b**) leaf number per pot (**c**) in two *M. officinalis* genotypes was assessed three weeks following drought stress. The standard error is represented by vertical bars, and statistically significant differences ($P < 0.01$) are indicated by different letters above the bars

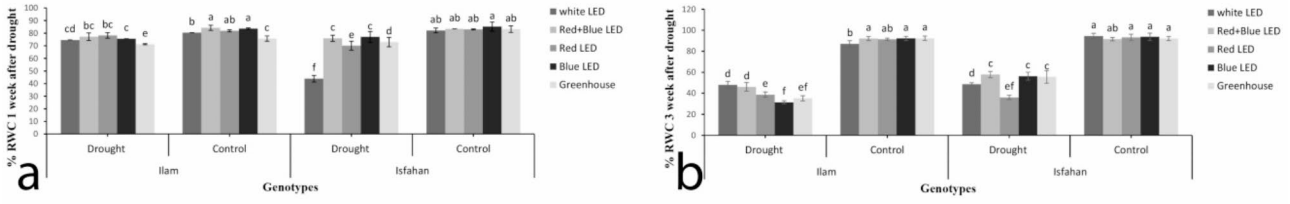

Fig. 2 The impact of LED light priming on RWC in two *M. officinalis* genotypes was assessed one week (**a**) and three weeks (**b**) following drought stress. The standard error is represented by vertical bars, and statistically significant differences ($P < 0.01$) are indicated by different letters above the bars

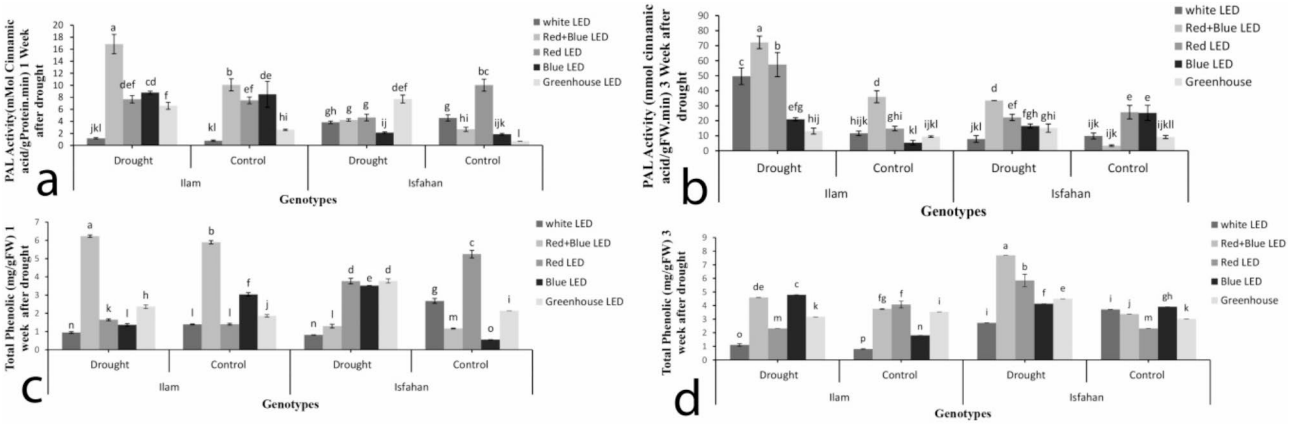

Fig. 3 The impact of LED light priming on PAL activity one (**a**) and three weeks following drought stress (**b**) and total phenolic one (**c**) and three weeks following drought stress (**d**). The standard error is represented by vertical bars, and statistically significant differences ($P < 0.01$) are indicated by different letters above the bars

from 1 to 3 weeks, indicating that the absence of drought helps maintain water retention. Isfahan generally retains a slightly higher RWC than Ilam under drought conditions, suggesting that it has better drought tolerance, especially after three weeks. Among the light treatments, white LED and red + blue LED tended to maintain higher RWC levels than other lighting options, especially at the 3-week point (Fig. 2a and b).

Impact of LED priming on phenolic and PAL activity at one and three weeks following drought stress

One week after drought stress, both Ilam and Isfahan genotypes exhibited the highest PAL activity under red + blue LED and greenhouse conditions. Under control conditions, red + blue LED and red LED treatments led to higher PAL activity in both the genotypes (Fig. 3a). After three weeks of drought stress, PAL activity remained significantly elevated, with the red + blue LED treatment consistently showing the highest activity in both Ilam and Isfahan. Notably, red + blue LED pre-treatment increased PAL activity by 5.5 times in Ilam and 2.2 times in Isfahan compared to greenhouse conditions (Fig. 3b). Over time, PAL activity under red + blue LED increased substantially, with a 4.3-fold increase in Ilam and a remarkable 7.9-fold increase in Isfahan between the first and third weeks of drought stress (Fig. 3c, d).

The highest levels total phenolic content was observed in the greenhouse and red + blue LED treatments one week after drought. Ilam showed a stronger response, with significantly higher phenolic content under these conditions compared to the blue LED and red LED treatments. Isfahan also responded well to greenhouse and red + blue LED treatments; though its phenolic content was generally lower than Ilam under drought conditions. As expected, phenolic levels were lower under control conditions compared to drought (Fig. 3c).

After three weeks of drought stress, the phenolic content remained elevated, with the red + blue LED treatment outperforming the others for both genotypes. While phenolic levels were stable but lower under control conditions, red + blue LED pre-treatment significantly boosted phenolic content, increasing it by 1.5 times in Ilam and 1.7 times in Isfahan compared to greenhouse conditions (Fig. 3d).

Impact of LED priming on anthocyanin levels at one and three weeks following drought stress

Drought stress, genotype, light treatment, and their interactions had a significant impact on anthocyanin levels ($P \leq 0.01$). During the first week, the Ilam genotype showed the highest anthocyanin content under white LED lighting. In contrast, the Isfahan genotype exhibited the highest anthocyanin levels under red + blue LED

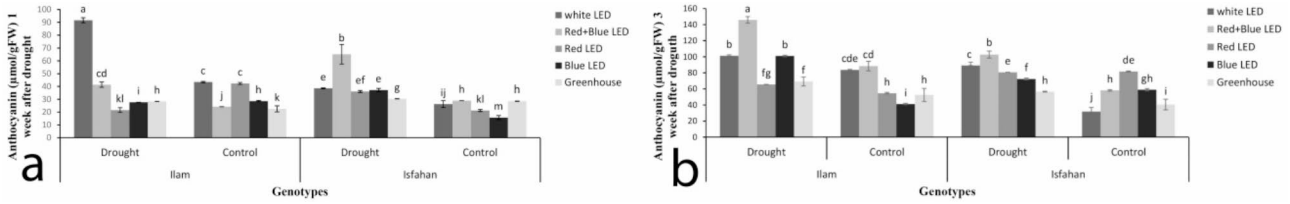

Fig. 4 The impact of LED light priming on anthocyanin in two *M. officinalis* genotypes was assessed one week (**a**) and three weeks (**b**) following drought stress. The standard error is represented by vertical bars, and statistically significant differences ($P < 0.01$) are indicated by different letters above the bars

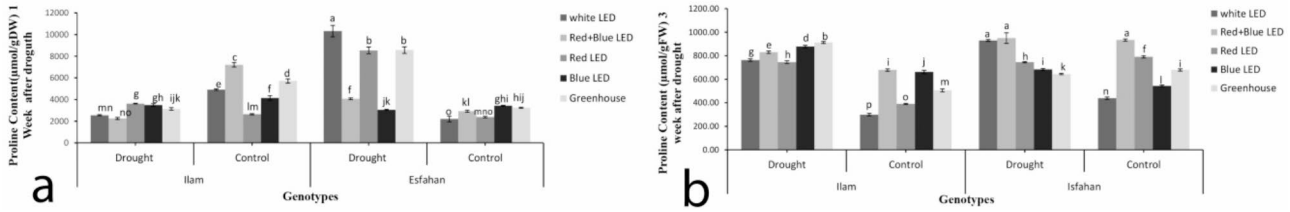

Fig. 5 The impact of LED light priming on proline in two *M. officinalis* genotypes was assessed one week (**a**) and three weeks (**b**) following drought stress. The standard error is represented by vertical bars, and statistically significant differences ($P < 0.01$) are indicated by different letters above the bars

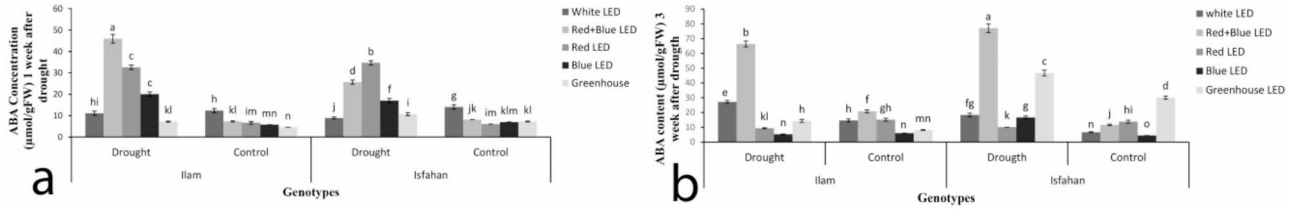

Fig. 6 The impact of LED light priming on ABA in two *M. officinalis* genotypes was assessed one week (**a**) and three weeks (**b**) following drought stress. The standard error is represented by vertical bars, and statistically significant differences ($P < 0.01$) are indicated by different letters above the bars

lighting when exposed to drought stress. Under control conditions, anthocyanin levels were generally lower in both genotypes than under drought conditions (Fig. 4a).

By the third week of drought stress, anthocyanin levels had increased significantly compared to those in the first week. Red + blue and white LED lights produced the highest anthocyanin levels in both genotypes under drought stress. Notably, anthocyanin levels increased 2.75 times in the Ilam genotype and 2 times in the Isfahan genotype between the first and third weeks (Fig. 4b).

Impact of LED priming on Prolin at one and three weeks following drought stress

Proline levels were significantly affected by drought stress, genotype, and light pre-treatment during both weeks of observation. In the first week, proline levels were moderately high under all lighting conditions, with the highest levels observed in the Ilam genotype under red LED lighting. Under control conditions, proline levels were generally lower than those under drought stress conditions. Red + blue LED lighting resulted in higher proline levels compared to the greenhouse and other lighting setups, whereas white LED lighting led to the highest proline accumulation in the Isfahan genotype. Among the control plants, greenhouse lighting resulted in the lowest proline accumulation (Fig. 5a).

By the third week, under drought stress, the highest proline content was observed under blue LED and greenhouse lighting, indicating that these conditions promote stress-related proline biosynthesis. Under control conditions, proline levels remained lower than those under drought stress. Red + blue LED lighting consistently resulted in relatively higher proline levels than other lighting setups, with the Isfahan genotype showing the highest proline content under red + blue and white LEDs. However, the overall proline accumulation under control conditions was lower than that under drought stress conditions. Notably, red + blue lighting consistently resulted in the lowest proline levels across conditions (Fig. 5b).

Impact of LED priming on ABA levels at one and three weeks following drought stress

The interaction between genotype, light, and drought stress significantly impacted ABA levels (Fig. 6). In the first week, drought stress increased ABA levels in both genotypes compared to controls. Red + blue and red LEDs produced the highest ABA levels under drought stress. The red + blue LED increased ABA by 6.5 and 2.5

times in the Ilam and Isfahan genotypes, respectively, compared to greenhouse light (Fig. 6a).

In the third week, red + blue, white, and greenhouse pre-treatments yielded the highest ABA levels under drought conditions. ABA levels increased under drought stress in both weeks, with the highest levels recorded for red + blue LED pre-treatment (Fig. 6b).

Discussion

This study found that fresh and dry weights of aerial parts and the number of leaves were higher in plants treated with LED lights compared to those grown under greenhouse lights. Under drought conditions, red + blue LED priming resulted in the highest leaf count, while red + blue and red LEDs increased fresh and dry weights compared to other treatments. Red + blue LED and red LED treatments are highly effective at boosting plant growth in both drought and normal conditions, making them promising options for reducing the impact of drought stress. While both Ilam and Isfahan followed similar trends, Ilam often had slightly higher growth metrics under both drought and control. Drought stress significantly reduced growth parameters in both genotypes across all light treatments, with leaf number reduction being a defensive mechanism against drought, as observed here. Drought stress severely hampers plant growth and development [1], reducing chlorophyll and carotenoid content, plant height, leaf area, and stem and root growth in basil, while increasing proline and carbohydrate levels [26]. Fresh weight reductions under drought, as seen in plants like *Asteriscus maritimus* [27] and *Albizia* [28], can result from inhibited cell expansion and reduced turgor pressure. The decrease in dry weight may stem from impaired growth, photosynthesis, and canopy structure, as observed in *Abelmoschus esculentum* [29].

Although drought stress reduced growth indices in this study, red + blue and red LED pre-treatments minimized these reductions, helping plants maintain growth under drought conditions. Bello and colleagues obtained similar results to the current study regarding the effect of LED light priming on stem fresh weight [30]. Similar negative effects of drought on growth indices have been documented in grapes [31], where it suppresses photosynthesis and growth by closing stomata [32]. This mechanism likely contributed to the reduced growth indices in lemon balm observed here. Drought-induced reductions in photosynthesis and assimilate production lead to early senescence and lower yields in maize. However, red + blue and red LED pre-treatments supported leaf retention, improving seedling growth under drought conditions. Blue and red LEDs are predominantly used for plant growth among narrow-spectrum lights because their wavelengths, around 460 nm and 660 nm respectively, are highly efficient for chlorophyll absorption, leading to optimal photosynthetic performance [33].

A decline in RWC is an early indicator of drought stress [7]. In this study, light, genotype, and drought stress significantly influenced RWC, both individually and interactively, one and three weeks after stress application. Drought reduced RWC in both genotypes across all light treatments, but red + blue and white LED pre-treatments in the Ilam genotype and red + blue and blue LED pre-treatments in the Isfahan genotype achieved the highest RWC levels under drought stress. Red + blue LED consistently maintained higher RWC, indicating improved drought resistance, as supported by Nxele et al. [34]. Priming with red or yellow LED light enhanced RWC under drought stress in research by Huang et al. [16]. Also, Cáceres-Cevallos showed similar results about thyme plants primed with LEDs under drought conditions [35].

Ilam shows stronger responses to drought in terms of both PAL activity and phenolic content. This suggests that it activates its defense mechanisms more robustly under stress, especially under favorable light treatments like red + blue LED. Red + blue LED provides the next best conditions under drought, with consistently high PAL activity and phenolic content. The combined spectrum may effectively stimulate stress responses in both genotypes. The accumulation of secondary metabolites is closely tied to environmental factors such as light, temperature, and nutrient availability [36]. Stress conditions, including drought, increase the concentration of secondary products like phenols and terpenes [37]. For instance, *Hypericum brasiliense* plants grown under drought showed higher phenolic content compared to controls [11]. This study similarly found increased phenolic levels three weeks after drought stress in plants primed with LED lights, particularly red + blue LEDs. Cáceres-Cevallos and colleagues observed that light priming positively affected the phenolic compounds in thyme, even under severe drought conditions [35].

PAL enzyme activity varied across light treatments, peaking in plants treated with red + blue LED one and three week post-drought stress. Enhanced PAL activity often correlates with higher phenolic content, as shown in cucumber seedlings treated with different light conditions [38]. This study confirmed a similar relationship between PAL activity and phenolic content in lemon balm under drought stress. Albergaria et al. [39] stated that medicinal plants increase the production of active compounds under drought stress, helping to protect against ROS and prevent damage to the photosynthetic apparatus. This could also be one of the defense mechanisms of primed lemon balm plants to survive drought stress.

Drought stress consistently results in higher anthocyanin accumulation compared to control conditions in both genotypes, indicating that anthocyanin biosynthesis is up regulated as a response to stress. The effects of different LED light treatments on anthocyanin production also vary. This study found that red + blue LED priming significantly enhanced anthocyanin levels in both genotypes at both one and three weeks after drought stress. Anthocyanin content was notably higher three weeks post-drought compared to one week, suggesting that the stress response intensifies over time. Additionally, the Ilam genotype demonstrated a greater capacity to accumulate anthocyanin under stress conditions compared to the Isfahan genotype, reflecting potential genetic differences in stress adaptation. Previous research shows that red LEDs promote phenolic compound accumulation, while red + blue LEDs enhance anthocyanin biosynthesis [40]. Phytochrome activation likely mediates this process by up regulating key enzymes such as PAL and chalcone synthase [41]. In this study, both PAL activity and anthocyanin levels increased significantly in plants treated with red + blue LEDs under drought conditions.

Proline is a marker of stress against UV-B damage and reduces cytosolic acidity under stress conditions [42]. In this study, proline content was found to be significantly higher under drought stress compared to control conditions, indicating that it serves as a biochemical marker for drought stress tolerance. Red + blue LED, white LED, and greenhouse lighting consistently led to greater proline accumulation under drought conditions in both genotypes. Proline levels tended to increase at three weeks compared to one week, likely reflecting a sustained response to prolonged drought stress. Additionally, the Isfahan genotype showed higher proline accumulation under drought stress than the Ilam genotype, particularly under white LED and red + blue LED lighting. This highlights potential genetic differences in drought tolerance mechanisms. Studies have shown that the wavelength of light affects the accumulation of proline; for example, proline was higher under blue LEDs than under white LEDs in tomato seedlings [42]. Rafeie et al. [43] discovered that basil plants pre-treated with LED light showed higher proline levels when exposed to high salinity, suggesting a beneficial effect of the light treatment in helping the plants cope with stress. Herein, proline increased more drastically during the first week of drought stress compared to the third week. Accordingly, the greatest efficiency of pre-treatments belonged to the Ilam genotype in the application of red and blue LEDs while in the Isfahan genotype, white LEDs. It supports the role of proline in osmotic balancing and its newly recognized function as an antioxidant.

The combination of red and blue LED light stands out as the most effective treatment for increasing ABA levels in plants under drought conditions for both genotypes. Between the two genotypes, *Isfahan* shows a stronger response to drought stress than *Ilam*, as indicated by higher ABA accumulation. Under normal (control) conditions, ABA levels remain low across all treatments, highlighting that this stress hormone is specifically triggered by water scarcity. As expected, both genotypes exhibit higher ABA concentrations during drought compared to control conditions. This is because ABA plays a key role in helping plants manage water stress by closing stomata to reduce water loss [44]. Interestingly, *Ilam* has a slightly lower ABA response under drought compared to *Isfahan*, suggesting that *Isfahan* may be better equipped to handle drought stress. ABA is a critical hormone in drought response, synthesized in roots and transported to aerial parts under water stress, inducing stomata closure and reducing water loss [3]. Drought stress triggers significant ABA accumulation in *Arabidopsis* leaves and roots [45]. Cáceres-Cevallos et al. [35] found that primed thyme plants had noticeably higher ABA levels than unprimed ones, across four different ecotypes (T4, T5, T6, and T7). This study found that drought stress increased ABA levels in both genotypes under LED pre-treatments compared to greenhouse light. Red + blue LED pre-treatment resulted in the highest ABA levels one and three weeks post-drought stress, further confirming the link between drought and ABA accumulation.

Conclusion

Results obtained from the present study indicate that red + blue LEDs had the most active pre-treatment role in mitigating potential drought stress effects in the growth, physiological, and biochemical characteristics of lemon balm genotypes. In contrast with the other LED groups, the highest values of fresh and dry weight, leaf number, RWC, phenolic content, as well as resistance markers such as proline and anthocyanin, under drought treatment were obtained in red + blue LEDs-primed plants. Under red + blue LED pre-treatment, the Ilam genotype resisted drought stress more than the Isfahan genotype, though both genotypes benefited from LED priming. These results suggest that priming lemon balm plants is a suitable and effective way to increase tolerance to drought stress. However, this increase in tolerance also depends on the type of plant, plant species, and other conditions. As observed in the results, some parameters measured under drought stress were higher in the Ilam genotype and some in the Isfahan genotype, which is due to genotypic differences.

Abbreviations

ABA	Abscisic acid
LED	light emitting diode
ROS	Reactive Oxygen Species

RWC Relative Water Content
FW Fresh Weight
DW Dry Weight
PAL Phenylalanine Ammonia Lyase

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Author contributions
Conceived and designed the experiments: Leila Shabani, Mohammad R. Sabzalian. Performed all experiments: TayebehAhmadi. Analyzed the data: Tayebeh Ahmadi, Leila Shabani, and Mohammad R. Sabzalian. Wrote the paper: Tayebeh Ahmadi, Leila Shabani, Mohammad R. Sabzalian, and Sahar Hassannejad. Edited the manuscript: Tayebeh Ahmadi, Sahar Hassannejad and Mehdi Rahimi.

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Declarations

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References

1. Hossain MA, Wani S, Bhattacharjee S, Burritt D, Tran L. Drought stress tolerance in plants, vol 1 physiology and biochemistry. Volume 1. Switzerland: Springer International Publishing Switzerland; 2016.
2. Ahmadi T, Shabani L, Sabzalian MR. Improvement in drought tolerance of lemon balm, Melissa officinalis L. under the pre-treatment of LED lighting. Plant physiol. Biochem. 2019;139:548–57.
3. Rahdari P, Hoseini SM. Drought stress: a review. Inter J Agron Plant Produc. 2012;3:443–6.
4. Ahmadi T, Shabani L, Sabzalian MR. LED light sources improved the essential oil components and antioxidant activity of two genotypes of lemon balm (*Melissa officinalis* L.). Bot. Stud. 2021;62(1):1–13.
5. Shahraki SH, Ahmadi T, Jamali B, Rahimi M. The biochemical and growth associated traits of Basil (*Ocimum Basilicum* L.) affected by silver nanoparticles and silver. BMC Plant Biol. 2024;24(1):92.
6. Ashraf M, Ozturkm M, Athar HR. Salinity and water stress, improving crop efficiency. The Netherlands: Springer; 2009.
7. Salehi-lisar SY, Motafakkerazad R, Hossain MM, Rahman IMM. Water stress in plants: causes, effects and responses, water stress. In: Ismail Md, Mofi zur R, editors. Water Stress. 2012:1–14.
8. Hongyun L, Xiangdong W, Donghui W, Zhirong Z, Zongsuo L. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* bunge. Ind Crops Prod. 2011;33:84–8.
9. De Abreu IN, Mazzafera P. Effect of water and temperature stress on the content of active constituents of *Hypericum Brasiliense* Choisy. Plant Physiol Biochem. 2005;43:241–8.
10. Liu H, Wang X, Wang D, Zou Z, Lianga Z. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* bunge. Ind Crops Prod. 2011;33:84–8.
11. Manukyan A. Effect of growing factors on productivity and quality of lemon catmint, lemon balm and Sage under soil less greenhouse production: I. drought stress. Med Aromat Plant Sci Biotechnol. 2011;5(2):119–25.
12. Abbasi Sh, Houshmand S, Ahmadi T. Volatile compositions and glandular trichomes of Zataria multiflora in different phenological stages under normal and drought stress conditions. BMC Plant Bio. 2024;24:483.
13. Chen K, Arora R. Priming memory invokes seed stress-tolerance. Environmen Experimen Bot. 2012;94:33–45.
14. Han SH, Park Y-J, Park C-M. Light priming of thermotolerance development in plants. Plant Sig Behav. 2018;14(1):1–3.
15. Poudel PR, Kataoka I, Mochioka R. Effect of red- and blue-light-emitting diodes on growth and morphogenesis of grapes. Plant Cell Tiss Organ Cult. 2008;92:147–53.
16. Huang L, Xiao Y, Wei L, Li Z, Li Y, Zhang X, Liao L, Wang D, Zhao X, Xiao Q, Guo Y. Drought tolerance of faba bean (*Vicia faba* L.) can be improved by specific LED light wavelengths. Photocynthethica. 2020;58(4):1040–52.
17. Silveira de Andrade R, Navroski MC, Pereira M, de O, Schatz Sa AC, Lencina KH. Light-emitting diodes (LEDs) in development and response to water stress in *Eucalyptus benthamii* seedlings (Myrtaceae). For Sci. 2023;53(11).
18. Ahmadi T, Shabani L, Sabzalian MR. Effects of LED light spectrum on growth and Rosmarinic acid content in Melissa officinalis L. Iran J Plant Proc Func. 2017;6(21):213–22.
19. Kadkhodaie A, Zahedi M, Razmjoo J, Pessaeaki M. Changes in some antioxidative enzymes and physiological indices among Sesame genotypes (*Sesamum indicum* L.) in response to soil water deficits under field conditions. Acta Physiol Plant. 2014;36:641–50.
20. Turner NC. Crop water deficits: a decade of progress. Adv Agron. 1986;39:1–51.
21. Singleton V, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144–58.
22. Morrison TA, Kessler JR, Hatfield RD, Buxton DR. Activity of two lignin biosynthesis enzymes during development of a maize internode. J Sci Food Agric. 1994;65(2):133–9.
23. Ishikura N, Hayashi K. Chromatographic separation and characterization of the components anthocyanins in radish root. Studies on anthocyanins. XXXVIII Bot Mag. 1963;76:6–13.
24. Troll W, Lindseley J. A photometric method for the determination of Prolin. J Biol Chem. 1954;215:655–60.
25. Kelen M, Demiralay EC, Sen S, Özkan G. Separation of abscisic acid, indole-3-acetic acid, Giberellic acid in 99 R (*Vitis berlandieri*, *Vitis rupestris*) and Rose oil (*Rosa damascena*) by reversed phase liquid chromatography. Turk J Chem. 2004;28:603–10.
26. Ghasemi Pirbalouti A, Malekpoor F, Salimi A, Golparvar A. Exogenous application of Chitosan on biochemical and physiological characteristics, phenolic content and antioxidant activity of two species of Basil (*Ocimum ciliatum* and *Ocimum Basilicum*) under reduced irrigation. Sci Hortic. 2017;217:114–22.
27. Rodriguez P, Torrecillas A, Morales MA, Ortuno MF, Blanco MJS. Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. Environ Experimen Bot. 2005;53:113–23.
28. Valli MVS, Paliwal K, Ruckmani A. Effect of water stress on photosynthesis, protein content and nitrate reductase activity of *Albizzia* seedlings. J Plant Biol. 2005;32:13–7.
29. Bhatt RM, Srinivasa-Rao NK. Influence of pod load on response of Okra to water stress. Indian J Plant Physiol. 2005;10:54–9.
30. Bello-Bello JJ, Martínez-Estrada E, Caamal H, Morales-Ramos V. Effect of LED light quality on in vitro shoot proliferation and growth of vanilla (*Vanilla planifolia* Andrews). Afr J Biotech. 2016;15(8):272–7.
31. Tooumi I, sehli M, Bourgou W, Jallouli S, Bensalem N, ghorbel A, mliki A. respone of ungrafted and grafted grapevine cultivars and rootstock (*Vitis sp.*) to water stress. J Int Sci Vigne Vin. 2007;41(2):85–93.
32. Chegah S, Chehrazi M, Albaj M. Effects of drought stress on growth and development *Frankenia* plant (*Frankenia Leavis*). Bulgarian J Agric Sci. 2013;19:659–65.
33. Gupta SD, Jatothu B. Fundamentals and applications of light emitting diodes (LEDs) in in vitro plant growth and morphogenesis. Plant Biotech Rep. 2013;7:211–20.
34. Nxele X, Klein A, Ndimba BK. Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants. S Afr J Bot. 2017;108:261–6.
35. Cáceres-Cevallos GJ, Quílez M, Albacete-Moreno AA, Jordán MJ. Does high-intensity light pre-treatment improve drought response in *Thymus Zygis* Ssp. *Gracilis*?? Plant Stress. 2024;11:100373.

36. Selmar D, Kleinwächter M. Stress enhances the synthesis of secondary plant products: the impact of stress-related over-reduction on the accumulation of natural products. Plant Cell Physiol. 2013;54(6):817–26.
37. Camm E, Towers N. Phenylalanine ammonia lyase. Phytochemistry. 1973;12:961–73.
38. Choi HG, Moon BY, Kang NJ. Effects of LED light on the production of strawberry during cultivation in a plastic greenhouse and in a growth chamber. Sci Hortic. 2015;189:22–31.
39. Albergaria ET, Oliveira AFM, Albuquerque UP. The effect of water defcit stress on the composition of phenolic compounds in medicinal plants. S Afr J Bot. 2020;131:12–7.
40. Alokam S, Li Y, Li W, Chinnappa CC, Reid DM. Photoregulation of phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) in the accumulation of anthocyanin in alpine and prairie ecotypes of *Stellaria longipes* under varied R/FR. Plant Physiol. 2002;116:531–8.
41. Carvalho R, Lattarulo M, Antune R. The role of phytochrome in stress tolerance. J Integr Plant Biol. 2011;53(12):920–9.
42. Kim K, Kook H, Jang Y, Lee W, Kamala-Kannan S, Lee K. The effect of blue-light-emitting diodes on antioxidant properties and resistance to *Botrytis cinerea* in tomato. Plant Pathol Microbiol. 2013;4:203–14.
43. Rafeie M, Shabani L, Sabzalian MR, Gharibi S. Pretreatment with leds regulates antioxidant capacity and polyphenolic profle in two genotypes of Basil under salinity stress. Protoplasma. 2022;259:1567–83.
44. Man D, Bao Y, Han L. Drought tolerance associated with proline and hormone metabolism in two tall fescue cultivars. Hortic Sci. 2011;46(7):1027–32.
45. Ikegami K, Okamoto M, Seo M, Koshiba T. Activation of abscisic acid biosynthesis in the leaves of *Arabidopsis thaliana* in response to water deficit. J Plant Res. 2009;122:235–43.

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