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Research article

Improvement in drought tolerance of lemon balm, *Melissa officinalis* L. under the pre-treatment of LED lighting

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ABSTRACT

Stress priming (pre-exposure of plants to various types of moderate stresses) could affect plant responses to subsequent severe stresses. Drought stress is one of the major threats to plants which reduces the global agricultural productions. Here we demonstrated that light emitting diodes (LEDs)-driven tolerant to drought stress in lemon balm plantlets was highly correlated with priming with these lighting sources. Plantlets of the two genotypes of *M. officinalis* L. were first grown in 4 incubators with different LED lamps, including white LEDs (380–760 nm), blue LEDs (460 nm), red LEDs (650 nm) and red + blue LEDs (70%:30%), in a greenhouse for 4 weeks. The potted plants were then subjected to drought stress. Under drought stress, LED-primed plants maintained significantly higher fresh and dry weight, relative water content (RWC), concentration of soluble sugars, antioxidant activity and higher content of proline, H_2O_2, abscisic acid (ABA) and rosmarinic acid than non-primed plants. The results of Real-Time RT-PCR confirmed that LED pretreatment up-regulated the expression levels of respiratory burst oxidase homologues (*RBOHs*) or NADPH oxidase, 9-cis epoxy carotenoid dioxygenase (*NCED*), and rosmarinic acid synthase (*RAS*), while down-regulated that of ABA 8′-hydroxylase (*ABA8Ox*). These findings suggest, for the first time, that pre-treatment of plants with red + blue LEDs could improve their growth and quality under drought stress.

1. Introduction

Melissa officinalis L. as a medicinal herb has been used in perfumes, cosmetics, tea and food products and it has also antibacterial and sedative effects. The leaves which contain polyphenolic compounds such as rosmarinic acid, trimeric compounds and some flavonoids are used in raw form in salad vegetables (Patora and Klimek, 2002). The major active ingredients in the leaves include phenolic acid, rosmarinic acid (RA), an ester of caffeic acid and 3,4-dihydroxphenyl lactic acid. Rosmarinic acid synthase (RAS; 4-coumaroyl-CoA: hydroxyphenyllactic acid hydroxycinnamoyltransferase) catalyses RA formation (Parnham and Kesselring, 1985). RA has antioxidant properties per se that increases the nutritional value and defense system of the plant.

Genetic and environmental factors have a tremendous effect on the amount of active ingredients of medicinal plants. The amount of essential oil or its chemical composition in lemon balm is also strongly influenced by factors such as light intensity, nutrition, temperature, plant's genotype and age and harvest time. For example, the amount of essential oil and tannin content has been reported to be increased with increasing light intensity from 1000 to 1500 lux (Turhan, 2006). Ozturk et al. (2004) reported that the amount of essential oil in lemon balm is positively affected by an increase in water deficit. This was negatively affected by rising salt concentrations. Therefore, it is expected that such factors will also affect the medicinal and nutritional properties of the plant. Many studies have also reported the effects of drought stress on accumulation of RA as well as its combined effect with UV-B radiation on photosynthetic capacity in rosemary plants (Luis and Johnson, 2005). It has been shown that UV-B irradiation increased caffeic acid, rosmarinic acid, narinjin and carnosic acid concentrations in irradiated rosemary plants, and the amount of rosmarinic acid was enhanced with increasing light intensity (Luis et al., 2007).

Drought stress is one of the major threats to plants, which reduces global yields of crops. Drought stress is described as reduction of water content, turgor loss, closure of stomata, as well as decrease in cell enlargement and growth of the plants. Reactive oxygen species (ROS) production in cell organelles is one of the disrupting factors that affects

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Abbreviations: ABA, Abscisic acid; LED, Light Emitting Diodes; RA, rosmarinic acid; ROS, Reactive oxygen species; RSA, radical scavenging activity

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growth and productivity of a drought-stressed plant. The excess ROS leads to the peroxidation of cell membrane lipids and destruction of enzymes, proteins and nucleic acid. Drought tolerance is a complicated mechanism that involves many physiological, biochemical and molecular responses, and equips plants to resist against the stress. Thus, various approaches are applied to improve plant growth and development under drought stress. Priming is known to be a potential method to increase stress tolerance, which is related to the stress memory (Bruce et al., 2007).

Cultivating plants in chambers or greenhouses with mild stress conditions can be considered as one of the applying ways of priming against drought stress (Langhans, 1978). Light emitting diodes (LED) are high intensity sources of visible radiation which are recently used in closed environments, such as greenhouses where blue, red, red + blue or white spectrums are dominantly illuminated, for growing horticultural and agronomic plants (Kurilcik et al., 2008). Morphological and physiological changes have been reported in many plants, such as spinach and lettuce (Yorio et al., 2001) and grapes (Poudel et al., 2008) following the treatment with various LEDs. Moreover, recent studies have reported changes in antioxidant activity, gene expression pattern and metabolism (Ma et al., 2012) of various plants which were exposed to LED wavelengths.

ABA is significantly increased in drought or salinity stress conditions and stimulates stomatal closure, gene expression alteration, and adaptive physiological responses (Kim et al., 2013). Moreover, ABA is known to be an important messenger that acts as a signaling mediator for regulating the adaptive responses of plants to different environmental stress conditions. However, there is little information on the effect of LED irradiation on endogenous ABA biosynthesis in plants. Currently, only one study has discussed the inductive role of red light emitting diodes on the concentration of ABA hormone in red grapes compared to those grown under blue LED condition and control. These results suggested that synthesis of endogenous ABA in the grape leaves may be associated with the action of phytochromes (Kondo et al., 2014).

LEDs may influence plants in different ways, such as alternation in growth properties, chemical compounds and physiological parameters. In addition, LED spectrums can induce considerable changes in plants at molecular level, especially when they are subjected to some repeating stressful factors, such as drought (or water stress). To survive under repeated stresses, plants are able to respond to subsequent stress events in a way which is different from that they first encounter (Avramova, 2015). However, this phenomenon has not been investigated in plants pre-treated with LED lights and subsequent drought stress exposure. Therefore, the aim of this study was to examine the long-term stress memory by 1) evaluating the effects of pre-treatment with LED radiation to prime the two genotypes of *M. officinalis* L. against drought stress, and 2) stimulating the accumulation of rosmarinic acid in plant tissues by LEDs irradiation and for production of lemon balm plants with better nutritional characteristics to be used in food and pharmaceutical industries.

2. Materials and methods

2.1. Plant materials and treatments

This experiment was carried out using a completely randomized design. The seedlings of *M. officinalis* were obtained from the fields of Ilam and Isfahan provinces, Iran. The uniform and healthy plantlets were cultivated in pots with 12 cm diameter containing sandy loam soil and were placed in greenhouse conditions. The mean temperature was 25–35 °C and the photoperiod was 16:8 light: dark in the greenhouse. After one month, the plantlets with 3–4 leaves were transferred to LED-array incubators. The incubators were programmed to provide an 16 h light/8 h dark photoperiod and photosynthetic photon flux density (PPFD) was maintained at 300 μmol/m^{2}s for all treatments. This was set by using different number of LED lamps per each LED panel. All the lemon balm plantlets were grown under five different light sources with a broad spectrum of white LEDs (380–760 nm), blue LEDs (460 nm), red LEDs (650 nm) and red + blue LEDs (70%:30%) as well as the greenhouse light as the control. After 4 weeks, the plantlets, which were maintained in the incubators with various LED irradiation, were transferred into the greenhouse. First, the relative soil moisture of the pots was evaluated (Kadkhodaie et al., 2014). To do this for the same period, all the pots (60 pots) were irrigated and then two hours after irrigation, the relative soil moisture content was recorded using a teta probe (AT Delta-T Devices SM300, Cambridge, England). The relative soil moisture was continuously recorded until it reached to 30%. For up to 3 weeks, half of the pots, three pots from each incubator (in overall, 30 pots as drought-treated pots), were irrigated with this specific interval time and the other half (30 pots, including 3 pots from each incubator as non-drought pots or controls) were irrigated when the soil moisture was 90% (supplementary file, S1). Sampling was performed to measure H_2O_2 and ABA concentrations as well as for gene expression analysis one week after drought stress treatment. Moreover, physiological and biochemical parameters were evaluated 3 weeks after drought stress treatment. The samples were immediately placed into liquid nitrogen and stored at −80 °C until further analysis.

2.2. Measurement of shoot fresh weight

After final harvesting, shoot fresh weight of plants at 3 replicates was measured. Each plant was weighed by using an electronic weighing device (JB1603-C/FACT, Switzerland) and weight of plants was determined as gram unit.

2.3. Measurement of relative water content (RWC)

To determine plant RWC, 3 leaves were weighed (fresh weight, FW) immediately after harvesting from the plant. The leaves were then placed in distilled water for 24 h at room temperature and then their turgid weights (TW) were measured. The samples were then dried in an oven at 110°C for 24 h to obtain their dry weights (DW). Relative water content was calculated by the following formula (Turner, 1986):

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

2.4. Measurement of soluble sugar concentration

Freeze-dried leaves (100 mg) were used for the determination of soluble sugar concentration. It was extracted in 5 ml 80% ethanol (v/v). After boiling the extracts and centrifuging them using anthrone reagent, absorption of samples was read at 625 nm (Porter and Villar, 1997).

2.5. Measurement of proline content

Proline content was estimated using the method of Bates et al. (1973). Extraction with 90% ethanol was performed. After centrifuging of samples at 14000*g for 5 min, 25 μl of supernatant was mixed with 475 μl of 70% (v/v) ethanol and 1 ml of reaction mixture (containing acetic acid and 96% (v/v) ethanol and ninhydrin and distilled water) and after centrifuging and boiling, proline content was measured by a spectrophotometer (Shimadzu UV 1601) at 520 nm and was calculated as μmol/g FW against standard proline.

2.6. Measurement of rosmarinic acid

The fresh and powdered aerial parts of *M. officinalis* were macerated with methanol (for 24 h at room temperature). After filtration, the solvent was evaporated in vacuum at 40 °C to obtain crude methanol extracts. Concentrated extracts were mixed with 1 ml 96% ethanol and passed from 0.22 µm filter. Rosmarinic acid content was estimated by the method of Öztürk et al. (2010).

2.7. Measurement of hydrogen peroxide content

Hydrogen peroxide content was determined based on a method previously described by Alexieva et al. (2001). The reaction mixture contained 0.1% TCA (w/v), 500 µl of the supernatant, K_2HPO_4 (pH = 7, 500 µl) and 1 M KI (1 ml). Absorbance readings of the samples were recorded at 390 nm. Hydrogen peroxide content was calculated using a standard curve based on the absorbance (at 390 nm) of H_2O_2 standards.

2.8. Measurement of scavenging activity using DPPH (RSA%)

The radical scavenging activity of the extracts was monitored using the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) following the method described by Braca et al. (2002) and Kulisic et al. (2004). Extract solutions (1 ml) were mixed with 1 ml of a freshly prepared DPPH solution (0.1 mM in methanol) and 3 ml of 96% ethanol. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark (until stable absorbance values were obtained). The reduction in the DPPH radical content was measured by monitoring the decrease of absorption at 517 nm. Radical scavenging activity of the extracts was calculated by the following formula:

% Radical scavenging activity = (control OD (optical density) - sample OD / control OD) $\times 100$

Methanol (80%) and DPPH solution (0.1 mM, 5 ml) were used separately as a blank and control sample, respectively.

2.9. Extraction and measurement of ABA

Two grams of leaf fresh tissues per sample was homogenized with 10 ml extraction solution containing butylated hydroxy-toluen (0.25 g), and ascorbic acid (0.24 g) dissolved in 90% methanol and stirred overnight at 4 °C. The extract was filtered through a Whatman filter and the methanol was evaporated under vacuum. The aqueous phase was adjusted to pH 8.5 with 0.1 M phosphate buffer and then partitioned with ethyl acetate 3 times. After removal of the ethyl acetate phase, pH of the aqueous phase was adjusted to 2.5 with 0.2 N HCl. The solution was partitioned with ethyl acetate 3 times, and then passed through andydrous sodium sulfate. After, the ethyl acetate phase was evaporated under vacuum and the dry residue containing hormones was dissolved in 0.5 ml of HPLC grade methanol and stored in vials at 4 °C. The chromatographic analysis was performed on a Waters HPLC (USA). This equipment has a Symmetry-C18 column (250 × 4.6 mm), UV detector (Waters 2487), mobile phase containing 0.2% acetic acid and 100% methanol (50:50 v/v) and the solvent flow rate in the column was 0.7 ml min^{-1}. The measurement and recording was done at 265 nm. Then, ABA concentrations were calculated using a standard curve prepared with known concentrations of (±)-abscisic acid (Sigma, A1049) (Kelen et al., 2004).

2.10. RNA extraction and cDNA synthesis

Total RNA was extracted from fresh leaf tissue of *M. officinalis* using DENA zist ASIA kit (DENAzist Asia Co., # S-1010-1, Iran) according to the manufacturer's instruction. To remove DNA contamination from extracted RNA samples, total RNA was treated with DNAase I enzyme using Takara Kit (Takara Bio, Inc., Otsu, Japan). The concentration of RNA was estimated via spectrophotometry. UV spectrophotometer (Ultrospec® 1100 pro, Amersham Pharmacia Biotech) was used for this purpose. Approximately 500 ng of total RNA from each sample was subsequently subjected to first strand cDNA synthesis using PrimeScript RT Enzyme Mix I enzyme (PrimeScript ™ RT reagent Kit, Takara company Inc., Otsu, Japan) according to the manufacturer's protocol. RNA extraction and cDNA synthesis were performed three times on each sampling date. The sequence of the rosmarinic acid synthase (*RAS*) gene was obtained from the NCBI database (GenBank accession no. FR670523.1). The primers of *NCED* (9-cis epoxy carotenoid dioxygenase) corresponding to the conserved regions of *NCED* were determined using a nucleotide alignment that included *NCED*s from a range of plant species: *Seasamum indicum* (XM-011084544.2), *Sesamum indicum* (XM-011099822.2), *Scutellaria baicalensis* (KC760149), *Fragaria x ananassa* (JX013945.1), *Fragaria vesca* (XM-004300619.2), *Erythranthe guttatus* (XM-012981017.1) and *Daucus carota* (NM-001329172.1). For ABA 8′-hydroxylase (*ABA8Ox*), also referred to as CYP707A, the ABA 8′-hydroxylase-f and ABA 8′-hydroxylase-r primers corresponding to the conserved regions of *ABAox2* were determined using a nucleotide alignment that included ABA 8′-hydroxylase 2 sequences from *Arabidopsis thaliana* (NM_123902), *Cajanus cajan* (XM_020346411), *Daucus carota* subsp. *sativus* (XM_017377913), *Medicago truncatula* (XM_003607923), *Salvia miltiorrhiza* (KP337746) and *Sesamum indicum* (LOC105170431) (XM_011091192). RNA-seq data (GSE100970) was used to design primers of *RBOH* gene (NADPH oxidase). The primer for beta-actin gene (Actin) was taken from Doring et al. (2014). The PCR primers used in this study were synthesized by Macrogen company (Korea). The primers were also designed using Geneious 4.8.5 software. Information on primers is presented in Table 1. After synthesis of cDNA from all the samples taken in the RT reaction, in order to determine the optimal interleaving temperature for primers, the proliferation of the desired products from the cDNA was carried out using thermocycler PCR gradient (Palm-Cycler). Finally, the appropriate annealing temperature for the real time reaction was also checked again. The PCR products were electrophoresed on 1% agarose gels in TBE buffer and visually quantified. Quantitative real-time PCR was performed using SYBR Premix Ex Taq TaKaRa (Takara Bio, Inc., Otsu, Japan) with a qReal-Time PCR using a Rotor-Gene Q instrument (Qiagene, Germany) following the instruction manual. Relative gene expression was also determined by qReal-Time PCR using a Rotor-Gene Q instrument (Qiagene, Germany). The PCR mixture (10 µl) contained 10 µM of each primer, 50 ng of the cDNA template and 5 µl of SYBR Premix Ex Taq TaKaRa (Takara Bio, Inc., Otsu, Japan). Relative expressions of the genes were analyzed using the formula $2^{-[\Delta\Delta Ct]}$ (Livak and Schmittgen, 2001). The purity of the amplified products was confirmed by melting curve analysis and agarose gel electrophoresis. All reactions were performed in triplicates.

2.11. Statistical analysis

All experiments described were performed at three replicates. For statistical analysis, factorial ANOVA test was used for all data, and means were compared according to the LSD test at 5 and 1% level of significance, using Excell and GraphPad Prism 6 software.

Table 1
The length of primers and products of the targeted genes.

Gene name	Primer sequence	Product length
Actin	Actin-f 5 -TGTATGTTGCCATCCAGGCCG-3 Actin-r 5 -AGCATGGGGAAGCGCATAACC-3	128
NCED	NCED-f 5 –CTGCGACTTGTTGTCCTA NCED-r 5 -TATGGTCTGGCTGTAGTG	128
ABA8Ox	ABAox2-f 5 –CCAACRCCTTCTTYTCCR ABAox2-r 5 -ATCATCACGCAMGGRCAYC	100
RAS	RAS-f 5 -ACGCCCCGACCTCAACCTTATC-3 RAS-r 5 -AAGTGGTGCTCGTTTGCCACG-3	128
RBOH	RBOH-f 5 -ATTGGAGCAATGGCGGCGCT-3 RBOH-r 5 -AGTGTTGCGGCACATCGGCA-3	159

Table 2
Analysis of variance (mean squares) of drought, genotype, light and their interactions on shoot fresh weight, shoot dry weight, RWC and proline, soluble sugars, RA, %RSA, H_2O_2, MDA, ABA concentration, and genes expression of *NCED*, *ABAox2*, *RAS* and *RBOH*.

Variable	Df	SFW	SDW	RWC	Proline	Soluble sugars	Rosmarinic acid	RSA%	H_2O_2	MDA	ABA	*NCED*	*ABAox2*	*RAS*	*RBOH*
Drought	1	2170**	8.3**	32555.3**	662931**	0.149**	0.528**	830.56**	16098319**	30772**	2513.95**	106.46**	1472**	12.1**	59.9 ^{ns}
Genotype	1	33.93**	0.34^{ns}	635.04**	57479**	0.142**	0.352**	479.87**	11765077**	316**	30.32**	35.86**	2867**	0.4**	4614.3**
Light	4	9.7**	209**	77.29**	83532**	0.022**	0.804**	361.13**	14575363**	7515**	433.94**	20.94**	3928**	15.7**	2622.9**
Genotype × Drought	4	5.6**	0.92^{ns}	299.96**	175245**	0.020**	0.059**	41.75*	474467**	6385**	62.92**	11.71**	6931**	0.025**	322**
Light × Drought	4	178**	4.4**	102.3**	74582**	0.012**	0.524**	160.19**	12401547**	5306**	638.63**	11.11**	8427**	4.9**	2206**
Genotype × Light	4	29.82**	4.4**	84.46**	72321**	0.029**	0.123**	99.55**	5050559**	2673**	71.92**	23.42**	8126**	1.04**	2498.9**
Genotype × Light × Drought	4	1.8**	12.86**	146.64**	30723**	0.029**	0.077**	273.02**	5015631**	4**	81.92**	14.34**	7231**	2.3**	4479.9**
Error	40	1.11	0.039	8.28	88.72	0.0022	0.0013	7.01	14140	22.01	0.66	0.084	0.83	0.0017	47.15

*, ** - significant difference at $P \le 0.05$ and $P \le 0.01$, respectively, ns, non-significant.

3. Results

3.1. Effects of LED lights and drought stress on SFW and SDW

ANOVA on the two genotypes of lemon balm pre-treated with the light spectra followed by drought stress are shown in Table 2. In control condition, the shoot fresh weight (SFW) and shoot dry weight (SDW) in both genotypes were the highest in red LED pre-treated group. Plants presented distinct growth responses to different light-quality pre-treatments and subsequent drought stress. SFW of the plantlets of both genotypes were significantly ($p \le 0.01$) decreased under drought stress compared to the non-drought conditions in all LED light pre-treated groups (Fig. 1, A). SFW of the two genotypes exposed to drought stress conditions were the highest in red + blue and red LED irradiation pre-treated groups, while it was the lowest in white LED light pre-treated group. The SFW values were significantly increased by 83% and 71% in response to red + blue LED light pre-treatment in plants obtained from Ilam and Isfahan (both genotypes), respectively, compared to the control group. The significant interactions between genotype, light, and drought stress on shoot dry weight are presented in Fig. 1, B. The SDW of Ilam genotype plants was significantly different in response to red + blue and blue pre-treatment and subsequent drought stress compared to that of the plants grown in non-drought condition. However, the SDW values were decreased in plants pre-treated with other LED wavelengths compared to the control group. In Isfahan genotype, the only significant difference in SDW was detected in the plants pre-treated with blue LED and subsequent drought stress, while the SDW values of the other pre-treated groups were not significantly different compared to those of the plants grown in non-drought condition. The highest shoot dry weight in this genotype belonged to the plants which were pre-treated with white and red LEDs.

3.2. Effects of LED lights and drought stress on RWC

The results of statistical analyses of relative water content of the two genotypes of *M. officinalis* are presented in Table 2 and Fig. 1, C. The LED pre-treatments did not induce any significant effect on RWC of both genotypes of lemon balm in comparison to the normal greenhouse condition. Drought stress induced a significant inhibitory effect on RWC in the leaves of both genotypes in all the pre-treated groups which were exposed to drought stress. In Ilam genotype, the highest RWC value was detected in the plants which were pre-treated with red + blue and white LEDs prior to being exposed to drought stress. In the plants of Isfahan genotype, the highest RWC values were detected in red + blue, and blue LED pre-treated and greenhouse groups. As a result, pre-treatment with red + blue LED light resulted in the highest amount of RWC and, therefore, resistance to water deficit in both genotypes of *M. officinalis*.

3.3. Effects of LED lights and drought stress on the content of soluble sugars

In the control condition, the concentration of soluble sugars in Ilam genotype was the highest in red + blue LED pre-treated group, while in Isfahan genotype, the highest concentrations of soluble sugar were detected in white and red LED pre-treated groups. The differences in soluble sugars concentrations of lemon balm (both genotypes) exposed to various light qualities and subsequent drought stress are presented in Fig. 2, A. Following drought stress, the amounts of soluble sugars were significantly higher in the plants of both genotypes which were pre-treated with red + blue LED.

3.4. Effects of LED lights and drought stress on proline content

The interaction between genotype, light, and drought stress significantly influenced the proline content of plants of lemon balm ($p \le 0.01$). Among all the pre-treated groups, red + blue LED

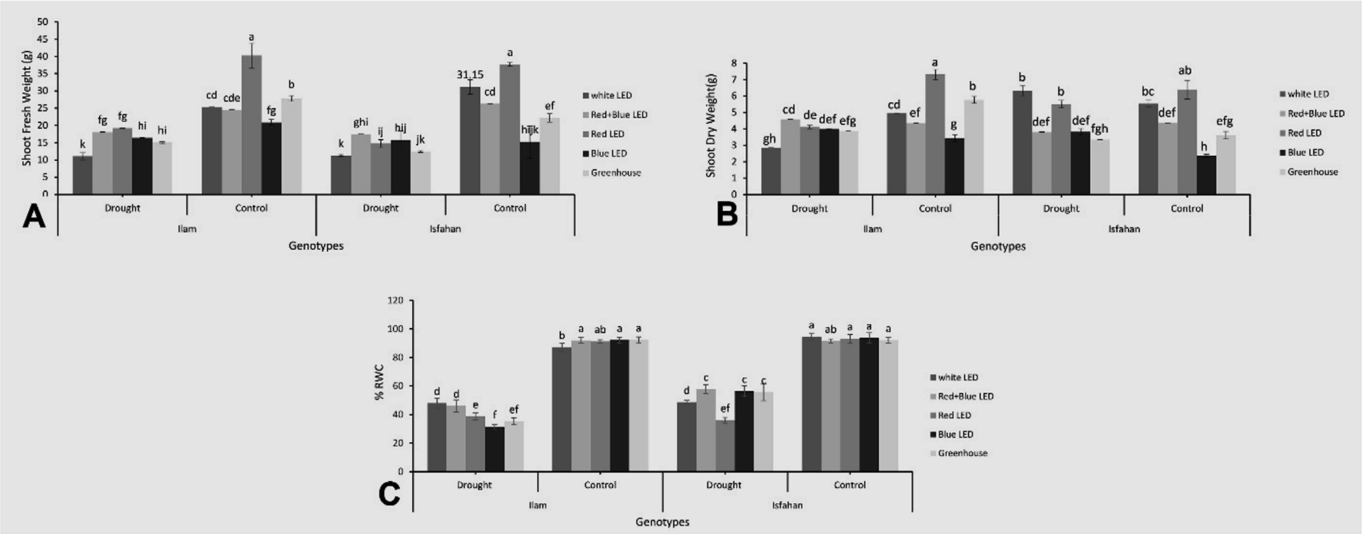

Fig. 1. Effect of drought on shoot fresh weight (A), shoot dry weight (B) and % RWC (C) in the two genotypes of *M. officinalis* grown under different light sources as pre-treatment. Vertical bars represent the standard error. Different letters above the bars indicate statistically significant differences ($P < 0.01$).

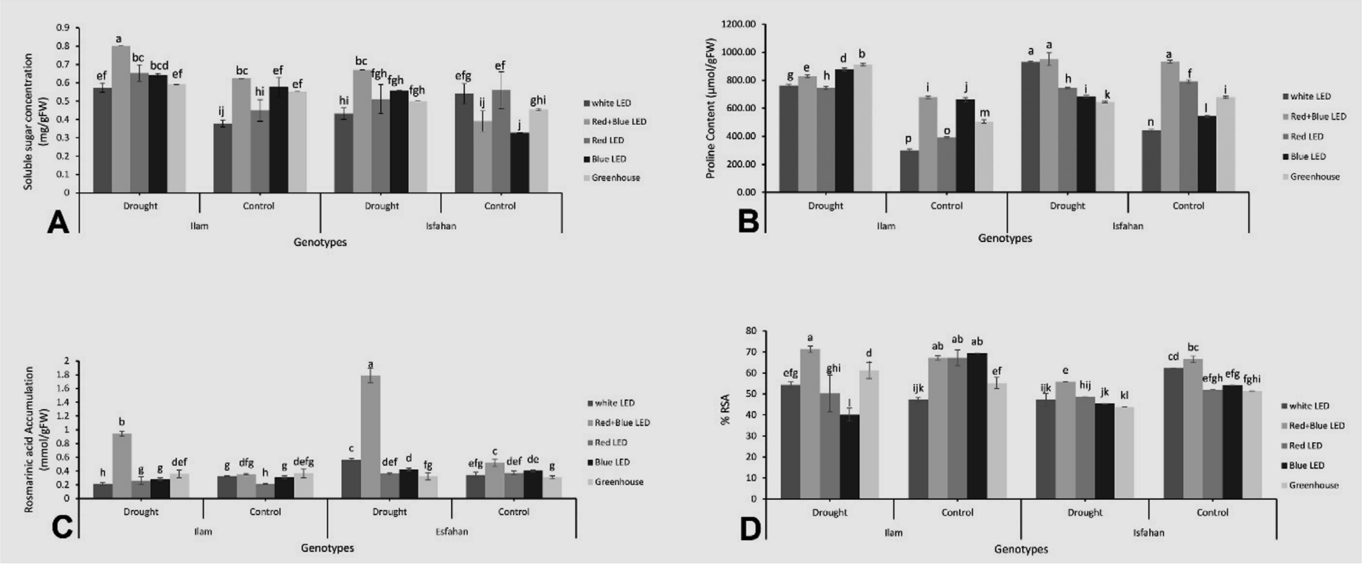

Fig. 2. Effect of drought on soluble sugar concentration (A), proline content (B), rosmarinic acid accumulation (C) and % RSA (D) in the two genotypes of *M. officinalis* with different light sources as pre-treatment. Vertical bars represent the standard error. Different letters above the bars indicate statistically significant differences ($P < 0.01$).

significantly increased the proline content of the two genotypes of *M. officinalis*. The leaf proline contents of both genotypes were increased in response to drought stress in all the pre-treated groups. In the plants of Ilam genotype, a significant decrease was observed in proline content in LED pre-treated plants, which were exposed to drought stress compared to those grown in the greenhouse. In other words, in Ilam genotype, the combination of LED lights and drought stress suppressed the proline accumulation. However, in the plants of Isfahan genotype, the combination of all LED pre-treatments and drought stress resulted in an increase in the proline content compared to the greenhouse light pre-treatment. Among the plants of this genotype, exposure to drought stress significantly increased the proline contents in red + blue and white LED pre-treated groups (Fig. 2, B) by 67 and 69%, respectively, compared to that of the control group.

3.5. Effects of LED lights and drought stress on rosmarinic acid accumulation

The effects of various light pre-treatments on rosmarinic acid (RA) accumulation of the leaves in both drought and control conditions depended on the type of the light (Fig. 2, C). The RA contents were equal in all the LED pre-treated groups, except the blue LED pre-treatment, in Ilam genotype, in the control condition. However, in Isfahan genotype, RA content was the highest in red + blue LED pre-treated group. In Ilam genotype, drought stress induced a decrease in RA level in white LED pre-treated group; however, both red + blue and red LED pre-treatments increased the RA contents. Hence, the amount of RA was not altered in blue LED and greenhouse light pre-treated groups compared to the respective controls. Red + blue LED pre-treatment in drought-stressed plantlets of Ilam genotype and Isfahan caused an increase of rosmarinic acid up to 2.62 and 5.58 times higher than controls. In contrast, in Isfahan genotype, the RA contents were not significantly changed in red and blue LED as well as the greenhouse light pre-treated groups. Interestingly, the interaction between red + blue LED and drought dramatically increased RA levels in plant leaves of both genotypes.

3.6. Effects of LED lights and drought stress on RSA%

In the present study, the ability of various extracts of the two genotypes of *M. officinalis* to quench DPPH free radical was investigated. Therefore, extracts obtained from *M. officinalis* leaves were subjected to a DPPH free radical scavenging assay. The LED pre-treatments, especially red + blue LEDs, increased RSA percentage in both genotypes. The results of this assay indicated that the plant responses to the combined LEDs light and drought stresses were distinctly different from those detected in the plants, which were not exposed to drought stress (Fig. 2, D). In Ilam genotype, drought stress significantly increased RSA percentage in white LED light pre-treated group compared to the control. Drought stress significantly decreased RSA percentage in both red and blue LED pre-treated groups compared to the control; however, no significant difference was observed in red + blue LED light pre-treated group. Similarly, in Isfahan genotype, drought stress reduced the radical scavenging activity in all LEDs pre-treated plants compared to their respective controls. Furthermore, in both genotypes, the highest free radical scavenging activity was observed in red + blue LED pre-treated plants.

3.7. Effects of LED lights and drought stress on the content of H_2O_2

The significant effect of the interaction between genotype, light, and drought stress on H_2O_2 content is presented in Fig. 3. In Ilam genotype, the greenhouse condition and in the other genotype, red and red + blue LED pre-treatments induced the highest increment in the production of H_2O_2. In the first week of post-drought treatment, in both genotypes, the intracellular levels of H_2O_2 were increased markedly in most of the LED pre-treated groups compared to the respective controls. In the plants exposed to drought stress, the maximum H_2O_2 contents were detected in red + blue LED light pre-treated groups of both genotypes. Drought-induced leaf H_2O_2 content was 1.18 folds higher in red + blue LED pre-treated group of Isfahan genotype than that of Ilam genotype.

3.8. Effects of LED lights and drought stress on ABA content

The endogenous ABA concentrations were increased in white LED pre-treated lemon balm compared to the other pre-treated groups of both genotypes in the control conditions (Fig. 4). Under drought stress conditions, ABA concentrations were significantly higher in red + blue LED pre-treated plants than those in the other light pre-treated groups of Ilam genotype. However, in Isfahan genotype, the endogenous ABA concentrations were higher in red LED pre-treated plantlets compared to the other pre-treated groups. In drought-stressed plants of Ilam genotype, concentration of ABA under LED pre-treatments ranged from 11.15 μmol to 45.94 μmol/gFW, whereas it was 7.22 μmol/gFW in the plants grown in greenhouse. In Isfahan genotype, the range of ABA concentration in the LED pre-treated groups, except for white LED, was from 17.05 μmol to 34.68 μmol/gFW, versus in the greenhouse grown plants, that it was 10.72 μmol/gFW.

3.9. Effects of LED lights and drought stress on the expression of NCED, ABA8Ox, RAS and RBOH genes

The interaction between genotype, light, and drought stress significantly affected the relative expression of *NCED* gene (Fig. 5, A). Evaluation of the relative expression of this gene in Ilam genotype revealed that drought stress significantly up-regulated the expression levels of *NCED* in all the pre-treated groups, except for blue LED light, compared to their respective controls. The highest expression level of *NCED* gene among the drought-stressed plants of this genotype was detected in red + blue LED pre-treated group (4.46 fold). In Isfahan genotype, interaction of drought stress and all the pre-treatments, except red LED light pre-treatment, resulted in an increased expression level of this gene compared to the controls. Among drought-stressed plants, those pre-treated with blue and red + blue LED presented an increase in the expression of *NCED* gene (11 and 6.57 folds, respectively) compared to drought-stressed plants under the greenhouse condition.

In the non-drought conditions, blue and red LED pre-treatments up-regulated the expression level of *ABA8Ox* gene in both Ilam and Isfahan plants. Evaluation of the effect of genotype, light, and drought stress interaction on the relative expression of *ABA8Ox* gene in the leaves of lemon plants revealed that the expression levels of this gene were up-regulated in Ilam genotype in white, red and red + blue LEDs pre-treated groups in comparison to the controls. Under drought stress, the highest expression level of *ABA8Ox* gene belonged to white LED pre-treated plantlets. Drought stress in the plants of Isfahan genotype pre-treated with red + blue and blue LEDs resulted in no change in the relative expression of *ABA8Ox* gene compared to the controls. The interaction of drought stress with white LEDs irradiation led to an increase in the expression level of this gene as compared to the non-drought controls (Fig. 5, B).

In Isfahan genotype of *M. officinalis*, the plants that were grown in the incubators containing red + blue LED lamps presented the highest expression level of *RAS*. Changes in the expression levels of *RAS* gene under the influence of drought stress were different in the plants exposed to different levels of light. The interaction of drought stress with red + blue LED irradiation increased the expression level of *RAS* gene in Ilam genotype. Also, interaction of drought stress with both white and red + blue LED irradiations in Isfahan genotype up-regulated the expression level of *RAS* gene. The highest expression level of this gene was found in the plants grown in the incubators with red + blue lamps

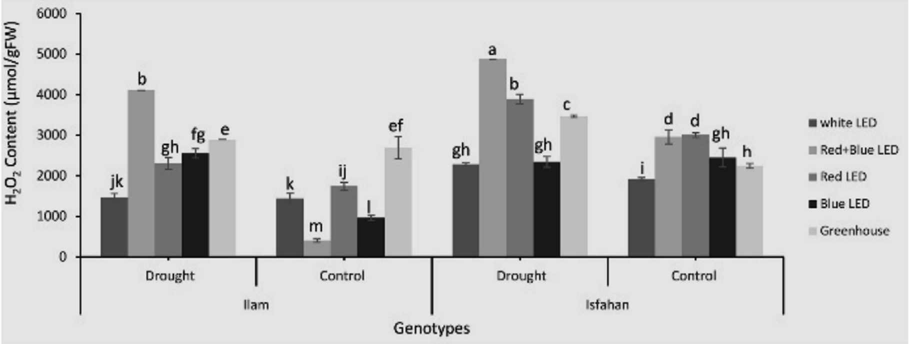

Fig. 3. Effect of drought on H_2O_2 content in the two genotypes of *M. officinalis* with different light sources as pre-treatment. Vertical bars represent the standard error. Different letters above the bars indicate statistically significant differences ($P < 0.01$).

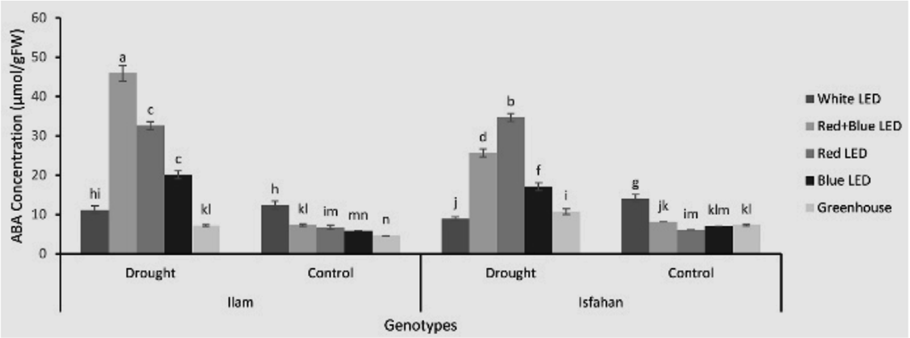

Fig. 4. Effect of drought on ABA concentration in the two genotypes of *M. officinalis* with different light sources as pre-treatment. Vertical bars represent the standard error. Different letters above the bars indicate statistically significant differences ($P < 0.01$).

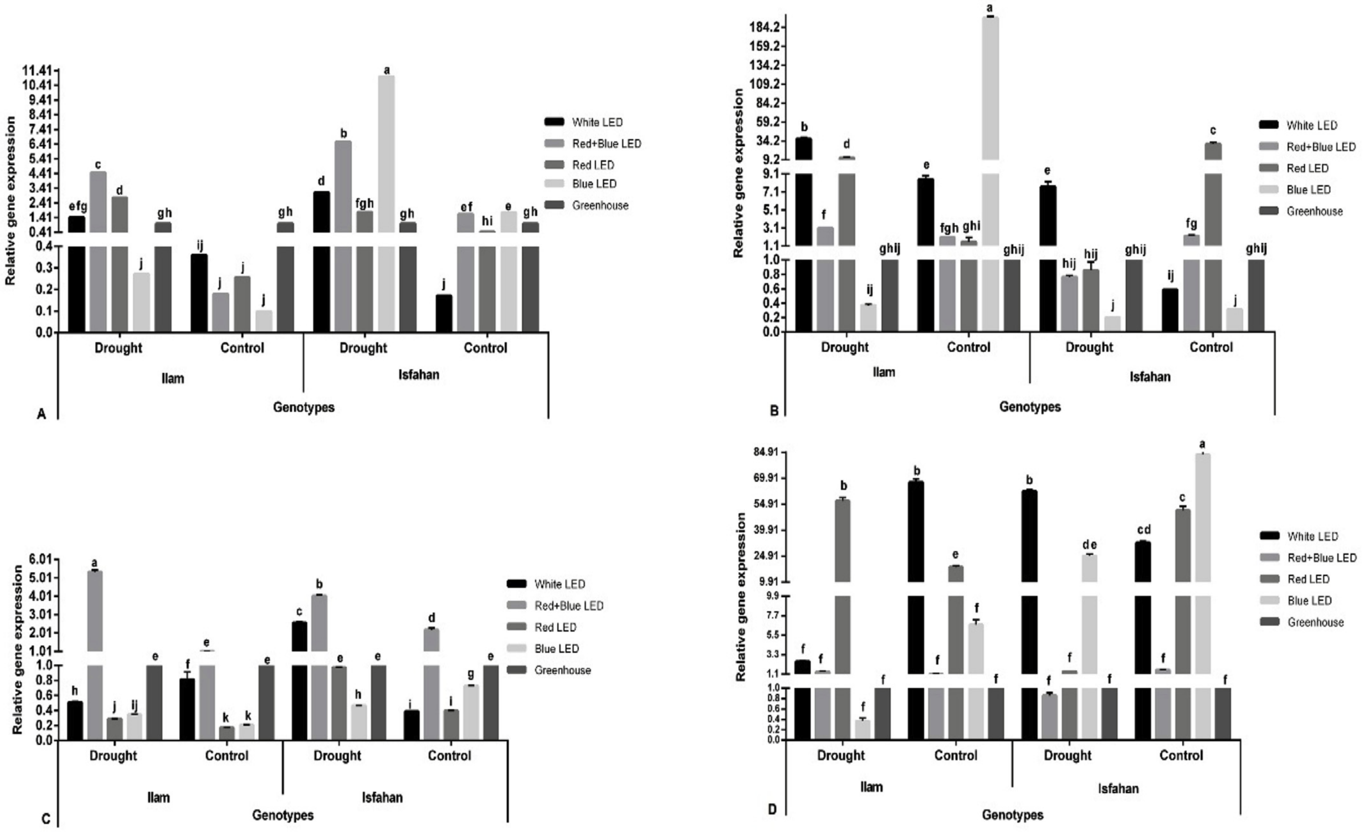

Fig. 5. Effect of drought on *NCED* (A), *ABA8Ox* (B), *RAS* (C) and *NADPH oxidase RBOH* (D) expression in the two genotypes of *M. officinalis* with different light sources as pre-treatment. Vertical bars represent the standard error. Different letters above the bars indicate statistically significant differences ($P < 0.01$).

(Fig. 5, C).

Expression of *RBOH* in control conditions in both Ilam and Isfahan genotypes was up-regulated in white and blue LED pre-treated groups. Pre-treatment of Ilam genotype with both white and blue LEDs, under drought stress, down-regulated the expression level of *RBOH* gene. However, *RBOH* gene was highly expressed in red LED pre-treated group compared to the control. Enhancement in the expression levels of *RBOH* was observed in white and blue LED pre-treatments under the drought conditions compared to the control in Isfahan genotype (Fig. 5, D).

4. Discussion

The results of the present study clearly demonstrated the specific role of LED irradiation in the priming of *M. officinalis* plantlets against drought stress. Our findings indicated that in both genotypes of *M. officinalis*, red LED pre-treatment induced the greatest effect on the fresh and dry weight of the plantlets in the control conditions. Considering the absorption level of CO_2 into the mesophilic intercellular spaces, red light irradiation played an important role in photosynthesis since it induced and thus stimulated the guard cells and produced the energy required to open the stomata by the photophosphorylation of the cells (Kostopoulou et al., 2010).

In drought stress and almost in both genotypes of lemon balm, it seemed that the effects of the combination of red and blue LEDs and red LED on the growth (fresh weight) of plantlets were statistically equal. However, the plantlets grown in the incubators containing red + blue LED lamps were healthier and more succulent than the other treatment groups, which probably indicates the complementary effect of blue light on the growth of this plant.

SDW values in Ilam genotype under drought conditions were higher in red + blue, red and blue LED pre-treated groups; however, the effects of white and red LED lamps on these characteristics were higher in Isfahan genotype. It has been shown that red LED spectrum is quite consistent with the absorption region of chlorophyll *a* and *b* in the chloroplasts of plants (Sabzalian et al., 2014). Moreover, it has been reported that blue light induces supplementary effect. Red light may be more involved in photosynthesis; however, in agreement with our findings, the results of a study conducted by Sabzalian et al. (2014) have indicated that neither pure red LEDs nor pure blue LEDs are sufficient for full and satisfactory mint growth. Therefore, plant species seem to be unable to accomplish their normal growth under pure red LEDs.

The RWC index in white and red + blue LED pre-treatments of Ilam genotype as well as in red + blue, blue LEDs and greenhouse pre-treatments of Isfahan genotype were increased under drought stress compared to those of the other pre-treatments. Under drought stress, leaf RWC plays an important role in plant tolerance to stress by inducing osmotic equilibrium due to the accumulation of osmoprotectants (Siddiqui et al., 2015). Siddiqui et al. (2015) have reported that the difference in RWC in various genotypes of *Vicia faba* under drought stress can be related to their ability to absorb water from soil. In the present study, RWC reduction was observed in all LED pre-treated groups of both lemon balm genotypes while applying drought treatment to the plantlets. However, the effect of LED pre-treatments of these two genotypes in maintaining the high level of RWC could be attributed to the effect of the differences in their characteristics; since they showed different responses to drought stress in different optical pre-treatments. The amount of RWC in the plant increases with resistance to drought. Therefore, it can be concluded that the plantlets of Ilam genotype pre-treated with red + blue and white LEDs are more resistant to drought due to their higher content of leaf water than the greenhouse grown plantlets. In Isfahan genotype, although red + blue and blue LED pre-treatments increased this indicator, the greenhouse pre-treatment response was similar to that of the two pre-treatments and was not significantly different.

We found that various light lamps induced different effects on the concentration of soluble sugars in the two genotypes, as reported by Kerepesi and Galiba (2000). In this study, red + blue LED pre-treatment of both genotypes under drought stress resulted in an increased concentration of soluble sugars. In fact, the LED lights induced significantly different effects on the metabolic systems of plants. Light changes not only affect sugar content, but also make them effective for vital processes as messengers. The soluble sugars play an active role in the regulation of growth, photosynthesis, carbon dividing, carbohydrate and lipid metabolism, osmotic homeostasis, protein synthesis and gene expression under abiotic stresses and are important for membrane stability. Increased concentration of soluble sugars, such as glucose, sucrose and fructose, enhances plant tolerance to abiotic stresses, such as drought, salinity and cold (Sami et al., 2016). Therefore, it seems that the other possible reason for the better growth of lemon balm plantlets in this research in red + blue LED pre-treatment group followed by drought stress could be the higher accumulations of soluble sugars.

The results of the present study indicated that proline contents of red + blue LED pre-treated plantlets of both Ilam and Isfahan genotypes were increased in control conditions (non-drought conditions). In order to investigate the effect of various light wavelengths on proline accumulation, Kim et al. (2013) have measured the amount of proline in leaf and stems of tomato grown exposed to various light wavelengths. They have found that various sources of light significantly altered the amounts of proline in seedlings of tomato, so that the accumulation of proline increased in leaves and stems of blue LEDs-exposed seedlings compared to that of those exposed to white LED. Significant increase of proline accumulation was also observed during the progressive increment of drought stress. The accumulated proline acts as an antioxidant and stabilizes macromolecules under drought stress (Man et al., 2011). Therefore, it is probable that accumulation of proline induced by LED light treatments, such as red + blue and white LEDs, in Isfahan genotype under drought may play a protective role against drought stress due to its physiological properties. However, no increase was detected in the proline content of LED pre-treated plantlets of Ilam genotype which were subjected to drought stress, indicating the differences in the performance of different genotypes, even within a plant species, against various stresses. In Isfahan genotype, the increased proline content is possibly related to the better growth and tolerance of red + blue LED pre-treated plantlets in the drought conditions.

Hydrogen peroxide (H_2O_2) is one of the most commonly studied ROS as a priming agent due to its greater stability and diffusion properties in plants. Based on the results of the present study, drought stress induced an increase in H_2O_2 accumulation in the LED light-exposed plants. Therefore, LED lamps and drought stress might increase H_2O_2 to act as a priming compound in order to facilitate the signaling processes involved in coping with different stresses.

In the two genotypes of *M. officinialis*, pre-treatment with red + blue LED and drought stress exposure led to an increase in H_2O_2 production. However, the expression of *RBOH* gene, a key enzyme in ROS production which is specialized respiratory burst oxidase homologues (*RBOHs*) or NADPH oxidase, was higher in the red LED pre-treated plants. Considering the various roles of H_2O_2 in different responsive mechanisms in plants, it can be concluded that high production of H_2O_2 in red + blue LED pre-treated plantlets, might be resulted in increasing plant resistance to drought stress in this pre-treatment. It has been demonstrated that ABA-induced production of H_2O_2 in rice leaves was established one day after the senescence of leaves (Hung and Kao, 2004). It can also be argued that ABA hormone might induce the increased H_2O_2 production in the lemon balm plantlets.

The results of this study demonstrated that the increase in the production of rosmarinic acid in lemon balm in response to the interaction of LEDs pre-treatments and drought stress was higher than that induced by LEDs pre-treatments or drought stress, individually. Therefore, the pre-treatment with red + blue LEDs resulted in increased production of rosmarinic acid under drought stress in both genotypes; moreover, these findings were confirmed by the up-regulated level of *RAS* gene in the red + blue LEDs pre-treated plants. Different studies have shown that LEDs, due to the high intensity of their photosynthetic photon flux, and the stimulation of receptors involved in the production of polyphenols and rosmarinic acid, would led to an increase in the production of these compounds (Iwai et al., 2010). In addition, artificial light irradiation could increase the amounts of caffeic acid in plants. The results of a study conducted by Iwai et al. (2010) have demonstrated that the combination of blue LED light, red laser light and UV-A increased the production of polyphenols in *Perilla frutescens* var. *purpurea* cv *Akajiso*. The authors have concluded that the increased levels of phenolic compounds might be closely regulated by blue light-induced light receptors, such as cryptochroms and phototropins (Iwai et al., 2010).

Several studies have reported the effect of drought stress on increasing the production of rosmarinic acid with ROS detoxifying effect (Trócsányi et al., 2015). Mittler (2002) has argued that drought stress induces ROS accumulation, which results in oxidative stress in plant cells, while plants can naturally metabolize it through an antioxidant system to remove and detoxify ROS. In this study, the red + blue LED pre-treated plantlets, which presented the highest amount of rosmarinic acid and antioxidant properties, were more resistant to drought stress possibly due to their higher capability to remove and scavenge the compounds harmful to the plant, such as ROS.

According to the results of the present study, antioxidant activity of both genotypes of *M. officinalis* was similar in response to different pre-treatments with LED lights and drought stress. In both genotypes, the red + blue LED pre-treated plantlets presented the highest antioxidant activity under drought stress. However, in a study performed by Wojciechowska et al. (2015), 100% red LED did not increase DPPH depletion compared to the control. It seems that the different LEDs induce different effects on free radical elimination activity. Plant antioxidant activity and scavenging ability of DPPH are related to the level of phenolic compounds, including rosmarinic acid (Wojciechowska et al., 2015) as well as the other compounds that have antioxidant activity, such as proline (Man et al., 2011). Therefore, the enhanced production of rosmarinic acid and proline in red + blue LED pre-treated plants may lead to higher antioxidant capacity of the lemon balm plants.

It was expected that the amount of endogenous ABA increases in the lemon balm plants exposed to light emitting diodes and then drought. The increase in the endogenous ABA concentrations was not similar in the two genotypes. The highest amount of endogenous ABA in Ilam genotype was detected in red + blue LEDs pre-treated plantlets under drought, while the highest levels in Isfahan genotype belonged to red and then red + blue LEDs pre-treated plants. In *Poa pratensis* L., the ABA contents of leaves were increased linearly in all cultivars, from 3 to 11 days post-exposure to drought stress (Wang et al., 2004). Furthermore, this difference in the concentration of ABA hormone in various genotypes of a plant has been reported by other studies (Man et al., 2011). Therefore, high ABA content could be another reason for the better growth and well resistance of the red + blue LEDs pre-treated plants of Ilam and Isfahan genotypes under the drought condition.

Endogenous accumulation of ABA is controlled by both its synthesis and catabolism. Evidence suggests that increases in ABA contents are partially resulted by the transient increases in the transcript abundance of the rate-limiting NCEDs. The major mechanism to inactivate ABA is mediated by the cytochrome P450 monooxygenase ABA 8′hydroxylase (*ABA8Ox*, also referred to as CYP707A) (Umezawa et al., 2006). Interaction of the two stresses also affected the expression of the genes involved in ABA biosynthesis and degradation. In our two *M. officinalis* L. genotypes, the expression levels of these genes were different. It has been reported that the expression level of *NCED* gene is affected by drought stress in different plants (He et al., 2016). Umezawa et al. (2006) have also reported the effect of drought stress on the expression levels of *CYP707A* genes in plants. Moreover, Ye et al. (2011) have demonstrated that up-regulation of *OsNCED3* and *OsABAox2* genes in rice leaf during water stress treatment were responsible for the accumulation and decline of ABA, respectively. Hence, the expression level of *VvNCED1* in grape plants treated with red LED was significantly higher than that of the untreated group. However, the expression of this gene in blue LEDs-treated plant was not significantly different to that of the untreated plants. The expression level of *VvCYP707A1* in plants treated with red LED was also significantly higher than that of the control plants on days 53, 77 and 99 after full flowering. Therefore, the increase in ABA content of red LED pre-treated grapes could be associated with the expression level of *VvNCED1*. Red light is absorbed by phytochrome, while blue light is absorbed by cryptochrome and phototropins. The results of the above-mentioned study indicated that endogenous ABA synthesis in leaves may be related to the function of phytochromes (Kondo et al., 2014).

In the present study, the expression levels of *NCED* and *ABA8Ox* genes were affected by drought stress, different radiation patterns of LEDs, as well as the two lemon balm genotypes. In both genotypes, almost all of the LEDs treatments increased the expression level of *NCED* gene in the plantlets that were under drought stress compared to those grown in the greenhouse light. Furthermore, up-regulated expression level of *ABA8Ox* gene was observed in both genotypes pre-treated with LEDs followed by drought stress exposure. Among the light pre-treated groups, *NCED* gene expression level was the highest in red + blue LED pre-treatment of Ilam genotype and blue LED pre-treatment of Isfahan genotype compared to the others treatments under the drought condition. The expression level of *ABA8Ox* gene was higher in white LED light pre-treated plantlets of both genotypes that were exposed to drought. Moreover, considering the expression levels of *NCED* and *ABA8Ox* genes, it can be concluded that ABA biosynthesis might be regulated by the changes in the expression levels of these two genes in the leaves of the plantlets. Therefore, it is reasonable to assume that high levels of ABA hormone production in red + blue LED pre-treated plantlets of Ilam genotype which were exposed to drought stress might be due to the up-regulated expression level of *NCED* gene, moreover, the down-regulated *ABA8Ox* gene expression of Isfahan genotype might be due to the reduced expression levels of *ABAOx2* in red and red + blue LED pre-treated plants.

Glucose plays a role in the activation of the genes involved in ABA biosynthesis as well as the suppression of the genes associated with ABA catabolism. In *Arabidopsis*, treatment of the plant with 167 mM glucose up-regulated the expression levels of the genes involved in ABA biosynthesis, such as *ABA2*, *ABI1*, and *ABI4*. In addition, higher glucose concentrations positively regulated the expression levels of the genes associated with ABA biosynthesis, such as *ABA2* and *NCED3* (Sami et al., 2016). Considering the results of this study which indicated the role of soluble sugars in the biosynthesis of ABA, it can be concluded that high concentrations of soluble sugars in red + blue LED pre-treated plantlets of Ilam genotype, especially under the drought conditions, might be resulted to the high levels production of this hormone.

5. Conclusion

As the results of this study indicate, LED lights, especially red + blue LEDs with a ratio of 70:30, effectively enhanced growth factors in lemon balm plantlets by improving the growth characteristics and augmentation of proline, as an osmoprotectant compound with antioxidant activity. Furthermore, the primed plants contained higher H_2O_2 levels (as a messenger that facilitates the better and faster transmission of messages in the plant), rosmarinic acid (as an antioxidant compound), and ABA hormone (that plays a key role in reducing the drought stress and water loss in plantlets under drought stress).

The amount of soluble sugars as the primary metabolite increased in the plantlets of lemon balm grown in incubators with red + blue LEDs in both genotypes. This also increases the potential of plantlets to synthesize other metabolites and improve their nutritional and medicinal properties. Also, under red + blue LED lamps, increasing the amount of soluble sugars in this study had a positive effect on increasing the amount of proline and rosmarinic acid, as an important and widely used compound in the pharmaceutical and cosmetic industries. Improvement of the antioxidant and ROS scavenging activity of the plantlets caused by the application of red + blue LEDs may raise the use of this herb to prevent oxidative damages to cells and to prevent the development of various diseases caused by the production of ROS. This will also increase its nutritional quality for the use in human diet and food industry. Therefore, LED sources can improve the ability of plants to withstand and resist under drought stress by improving their morphological, physiological and molecular characteristics. With due attention to some of the variations between the two genotypes of *M. officinalis*, application of red + blue LEDs is suitable for increasing the quality of lemon balm plantlets and its better resistance against drought stress.

Conflicts of interest

The authors declare that there is no conflict of interest.

Author contributions

Conceived and designed the experiments: Leila Shabani, Mohammad R. Sabzalian. Performed the experiments: Tayebeh Ahmadi. Analyzed the data: Tayebeh Ahmadi, Leila Shabani, Mohammad R. Sabzalian. Wrote the paper: Tayebeh Ahmadi, Leila Shabani, Mohammad R. Sabzalian. Edited the manuscript: Leila Shabani, Mohammad R. Sabzalian.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.04.021.

References

Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 24, 1337–1344.

Avramova, Z., 2015. Transcriptional 'memory' of a stress; transient chromatin and memory (epigenetic) marks at stress response genes. Plant J. 83, 149–159.

Bates, L., Waldren, R., Teare, I., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.

Braca, A., Sortino, C., Politi, M., Morelli, I., Mendez, J., 2002. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J. Ethnopharmacol. 79, 379–381.

Bruce, T.J.A., Matthes, M.C., Napier, J.A., Pickett, J.A., 2007. Stressful "memories" of plants: evidence and possible mechanisms. Plant Sci. 173, 603–638.

Doring, A.S., Pellegrini, E., Campanella, A., Trivellini, A., Gennai, C., Petersen, M., 2014. How sensitive is *Melissa officinalis* to realistic ozone concentrations? Plant Physiol. Biochem. 74, 156–164.

He, Zh, Zhan, Y., Zeng, F., Zhao, X., Wang, X., 2016. Drought physiology and gene expression characteristics of *Fraxinus* interspecific hybrids. Plant Growth Regul. 78, 179–193.

Hung, K.T., Kao, C.H., 2004. Hydrogen peroxide is necessary for abscisic acid-induced senescence of rice leaves. J. Plant Physiol. 161, 1347–1357.

Iwai, M., Ohta, M., Tsuchiya, H., Suzuki, T., 2010. Enhanced accumulation of caffeic acid, rosmarinic acid and luteolin-glucoside in red *Perilla* cultivated under red diode laser and blue LED illumination followed by UV-A irradiation. J. Funct. Foods. 2, 66–70.

Kadkhodaie, A., Zahedi, M., Razmjoo, J., Pessarakli, M., 2014. Changes in some antioxidative enzymes and physiological indices among sesame genotypes (*Sesamum indicum* L.) in response to soil water deficits under field conditions. Acta Physil. Plant. 36, 641–650.

Kelen, M., Demiralay, E., Şen, S., Özkan, G., 2004. Separation of Abscisic acid, Indole-3-Acetic acid, Gibberellic acid in 99 R (*Vitis berlandieri* x *Vitis rupestris*) and Rose Oil (*Rosa damascene* Mill.) by reversed phase liquid chromatography. Turk. J. Chem. 28, 603–610.

Kerepesi, I., Galiba, G., 2000. Osmotic and salt stress-induced alteration in soluble carbohydrates content in wheat seedlings. Crop Sci. 40, 482–487.

Kim, K., Kook, H., Jang, Y.J., Lee, W.H., Kamala-Kannan, S., Chae, J.C.H., Lee, K.J., 2013. The effect of blue-light-emitting diodes on antioxidant properties and resistance to *Botrytis cinerea* in Tomato. J. Plant Pathol. Microbiol. 4, 203.

Kondo, S., Tomiyama, H., Rodyoung, A., Okawa, K., Ohara, H., Sugaya, S., Terahara, N., Hirai, N., 2014. Abscisic acid metabolism and anthocyanin synthesis in grape skin are affected by light emitting diode (LED) irradiation at night. J. Plant Physiol. 171, 823–829.

Kostopoulou, P., Dini-Papanastasi, O., Radoglou, K., 2010. Density and substrate effects on morphological and physiological parameters of plant stock material of four forest species grown in mini-plugs. Scand. J. For. Res. 25, 10–17.

Kulisic, T., Radonic, A., Katalinic, V., Milos, M., 2004. Use of different method for testing antioxidative activity of oregano essential oil. Food Chem. 85, 633–640.

Kurilcik, A., Miklusyte-Canova, R., Dapkuniene, S., Zilinskaite, S., Kurilcik, G., Tamulaitis, G., Duchovskisand, P., Zukauskas, A., 2008. In vitro culture of *Chrysanthemum* plantlets using light-emitting diodes. Cent. Eur. J. Biol. 2, 161–167.

Langhans, R.W., 1978. A Growth Chamber Manual. Environmental Control for Plants, first ed. Comstock Publishing Associates, Ithaca, New York.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 25, 402–408.

Luis, J.C., Johnson, C.B., 2005. Seasonal variations of rosmarinic and carnosic acids in rosemary extracts. Analysis of them in vitro antiradical activity. Span. J. Agric. Res. 3, 106–112.

Luis, J.C., Pérez, R.M., González, F.V., 2007. UV-B radiation effects on foliar concentrations of rosmarinic and carnosic acids in rosemary plants. Food Chem. 101, 1211–1215.

Ma, G., Zhang, L., Kato, M., Yamawaki, K., Kiriiwa, Y., Yahata, M., Ikoma, Y., Matsumoto, H., 2012. Effect of blue and red LED light irradiation on cryptoxanthin accumulation in the flavedo of *Citrus* Fruits. J. Agric. Food Chem. 60, 197–201.

Man, D., Bao, Y., Han, L., 2011. Drought tolerance associated with proline and hormone metabolism in two tall fescue cultivars. Hortscience 46, 1027–1032.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.

Ozturk, A., Unlukara, A., Ipek, A., Gurbuz, B., 2004. Effects of salt stress and water deficet on plant growth and essential oil content of Lemon Balm (*Melissa officinalis* L.). Pak. J. Bot. 36, 787–792.

Öztürk, M., Duru, M.E., Ince, B., Harmandar, M., Topcu, G., 2010. A new rapid spectrophotometric method to determine the rosmarinic acid level in plant extracts. Food Chem. 123, 1352–1356.

Parnham, M.J., Kesselring, K., 1985. Rosmarinic acid. Drugs Future 10, 756–757.

Patora, J., Klimek, B., 2002. Flavonoids from lemon balm (*Melissa officinalis* L., *Lamiaceae*). Acta Pol. Pharm. 59, 139–143.

Porter, H., Villar, R., 1997. The fate of acquired carbon in plants: chemical composition and construction costs. In: Bazzaz, F.A., Grace, J. (Eds.), Plant Resource Allocation. Academic Press, San Diego, pp. 30–72.

Poudel, P.R., Kataoka, I., Mochioka, R., 2008. Effect of red-and blue-light-emitting diodes on growth and morphogenesis of grapes. Plant Cell Tissue Organ Cult. 92, 147–153.

Sabzalian, M.R., Heydarizadeh, P., Zahedi, M., Boroomand, A., Agharokh, M., Sahba, M.R., Schoefs, B., 2014. High performance of vegetables, flowers, and medicinal plants in a red-blue LED incubator for indoor plant production. Agron. Sustain. Dev. 34, 879–886.

Sami, F., Yusuf, M., Faizan, M., Faraz, A., Hayat, S.H., 2016. Role of sugars under abiotic stress. Plant Physiol. Biotech. 109, 54–61.

Siddiqui, M., Al-Khaishany, M., Al-Qutami, M., Al-whaibi, M., Grover, A., Ali, H., Al-Wahibi, M., Bukhari, N., 2015. Response of different genotypes of Faba bean plant to drought stress. Int. J. Mol. Sci. 16, 10214–10227.

Trócsányi, E., György, Z., Inotai, K., Szabó, K., Pluhár, Z., Radácsi, P., Németh, Z., 2015. Augus. Enhanced rosmarinic acid accumulation and rosmarinic acid synthase gene expression under drought stress in thyme *(Thymus vulgaris)*. In: Poster Session Presentation at the 63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Budapest.

Turhan, M., 2006. Melissa officinalis. In: Peter, K.V. (Ed.), Hand Book of Herbs and Species. Woodhead Publishing, Cambridge, pp. 390–399.

Turner, N.C., 1986. Crop water deficits: a decade of progress. Adv. Agron. 39, 1–51.

Umezawa, T., Okamoto, M., Kushiro, T., Nambara, E., Oono, Y., Seki, M., Kobayashi, M., Koshiba, T., Kamiya, Y., Shinozaki, K., 2006. *CYP707A3*, a major ABA 8´-hydroxylase involved in dehydration and rehydration response in *Arabidopsis thaliana*. Plant J. 46, 171–182.

Wang, Z.H., Huang, B., Bonos, S., Meyer, W., 2004. Abscisic acid accumulation in relation to drought tolerance in Kentucky Bluegrass. Hortscience 39, 1133–1137.

Wojciechowska, R., Długosz-Grochowska, O., Kolton, A., Żupnik, M., 2015. Effects of LED supplemental lighting on yield and some quality parameters of lamb's lettuce grown in two winter cycles. Sci. Hortic. 187, 80–86.

Ye, N., Zhu, G., Liu, Y., Li, Y., Zhang, J., 2011. ABA controls H_2O_2 accumulation through the induction of *OsCATB* in rice leaves under water stress. Plant Cell Physiol. 52 (4), 689–698.

Yorio, N.C., Goins, G.D., Kagie, H.R., Wheeler, R.M., Sager, J.C., 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. Hortscience 36, 380–383.