



Article

Combined Effect of Salinity and LED Lights on the Yield and Quality of Purslane (*Portulaca oleracea* L.) Microgreens

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Abstract: The present work aims to explore the potential to improve quality of purslane microgreens by combining water salinity and LED lighting during their cultivation. Purslane plants were grown in a growth chamber with light insulated compartments, under different lighting sources on a 16 h d^{-1} photoperiod—fluorescent lamps (FL) and two LED treatments, including a red and blue (RB)) spectrum and a red, blue and far red (RB+IR) LED lights spectrum—while providing all of them a light intensity of 150 μ mol m⁻² s⁻¹. Plants were exposed to two salinity treatments, by adding 0 or 80 mM NaCl. Biomass, cation and anions, total phenolics (TPC) and flavonoids content (TFC), total antioxidant capacity (TAC), total chlorophylls (Chl) and carotenoids content (Car) and fatty acids were determined. The results showed that yield was increased by 21% both in RB and RB+FR lights compared to FL and in salinity compared to non-salinity conditions. The nitrate content was reduced by 81% and 91% when microgreens were grown under RB and RB+FR, respectively, as compared to FL light, and by 9.5% under saline conditions as compared with non-salinity conditions. The lowest oxalate contents were obtained with the combinations of RB or RB+FR lighting and salinity. The content of Cl and Na in the leaves were also reduced when microgreens were grown under RB and RB+FR lights under saline conditions. Microgreens grown under RB light reached the highest TPC, while salinity reduced TFC, Chl and Car. Finally, the fatty acid content was not affected by light or salinity, but these factors slightly influenced their composition. It is concluded that the use of RB and RB+FR lights in saline conditions is of potential use in purslane microgreens production, since it improves the yield and quality of the product, reducing the content of anti-nutritional compounds.

Keywords: minerals; fatty acids; oxalate; nitrate; phytochemicals; antioxidants



Citation: Giménez, A.;
Martínez-Ballesta, M.d.C.;
Egea-Gilabert, C.; Gómez, P.A.;
Artés-Hernández, F.; Pennisi, G.;
Orsini, F.; Crepaldi, A.; Fernández,
J.A. Combined Effect of Salinity and
LED Lights on the Yield and Quality
of Purslane (*Portulaca oleracea* L.)
Microgreens. *Horticulturae* 2021, 7,
180. https://doi.org/10.3390/
horticulturae7070180

Academic Editor: Lilian Schmidt

Received: 8 June 2021 Accepted: 25 June 2021 Published: 3 July 2021

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1. Introduction

Since antiquity, purslane (*Portulaca oleracea* L.) has been used as a medicinal and edible plant. As an edible plant, the most frequent use of purslane is raw in mixed salads, being appreciated for its succulence and pleasant, slightly acidic taste [1]. Its morphological and nutritional characteristic makes it suitable as a ready-to-eat product that can be easily adjusted to new market requirements [2]. Furthermore, it presents a higher nutritional value as compared with other major cultivated vegetables, thanks to the numerous bioprotective compounds such as antioxidants and vitamins, essential amino acids, omega-3 fatty acids and several minerals, including potassium [3]. On the other hand, its acceptance in the human diet could be conditioned by the large amounts of some anti-nutritional compounds it contains, mainly oxalates and nitrates, an aspect to be considered in the

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cultivar selection [2] and ameliorated by the appropriate choice of the growing season [4]. In recent years, together with the rise in its consumption, an increase in the agricultural area devoted to this crop has been also observed, particularly in the Mediterranean basin [5].

Purslane has been rated as moderately tolerant to salinity [6] by accumulating salt in the tissues [7]. In general, purslane plants exposed to salinity stress were shown to increase the concentration of nutritional compounds, including total phenol contents (TPC), total flavonoid contents (TFC) and ferric reducing antioxidant power [8]. Moreover, leaf nitrate concentration was decreased in purslane plants grown under salinity above 5.0 dS m⁻¹ in the nutrient solution [9]. When purslane plants were treated with moderate salinity (up to 40 mM of NaCl in nutrient solution), no detrimental effects were observed in the biomass production, while an increase in the major fatty acids (linoleic, linolenic and palmitic acid) and a decrease in oxalic acid contents was measured [10]. Furthermore, plants exposed to four levels of salinity (60, 90, 120 and 240 mM NaCl) in the root zone for 40 days showed no signs of toxicity [11]. However, different accessions exhibit diverse performances under exposure to different levels of salinity stress, with evidence of ornamental purslane being generally more salt tolerant than common purslane [12].

Light-emitting diodes (LEDs) are increasingly adopted to efficiently provide lighting for production of several vegetable modalities and for quality preservation during storage, with proven potentialities to influence the metabolic pathways and therefore the biosynthesis of several bioactive compounds [13–15]. Fully controlled growing environments with artificial lighting (e.g., plant factories, vertical farms, growing chambers) are commonly used for microgreens cultivation. In these environments, light parameters (e.g., spectral quality, light intensity, photoperiod) can be optimized in order to enhance the nutritional value of microgreens [16,17]. Particularly, responses in terms of crop productivity and functional quality to changes in light spectrum depends on plant species [18], and for new and emerging species, including purslane, these effects are not yet well established. Recently, Kyriacou et al. [19] studied the effects of different spectral bandwidths on the nutritive and phytochemical composition of purslane, demonstrating that the use of monochromatic blue or red light resulted in higher accumulation of nitrate and lower biosynthesis of total polyphenols as compared to a combination of red and blue light. However, to our knowledge, no published information is available about the combined effects of salinity and LED spectral quality on the bioactive compounds' accumulation of purslane microgreens. Therefore, the aim of this study was to analyze the effects of salinity (80 mM NaCl) and LED spectral quality on yield and quality of purslane microgreens production. For that, biomass production, cations and anions content, fatty acids, and the main total bioactive compounds contents were determined in untreated and salt-treated plants grown under fluorescent light or two LED lighting treatments.

2. Materials and Methods

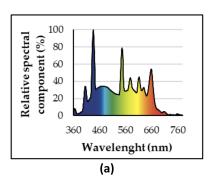
2.1. Plant Material and Growing Conditions

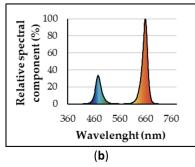
Seeds of common purslane (*Portulaca oleracea* L.), "Summer Purslane" (Tozer Seeds Ltd., Cobham, UK), were soaked in 0.5% sodium hypochlorite for 3 h under aeration and rinsed three times in distilled water. After sterilization, the seeds were evenly spread in 10×15 cm trays (0.3 g per tray) lined with autoclaved cellulose growth pads of white viscose (CN Seeds, Ely, UK), and immediately placed in three separated compartments for each light treatment in a plant growth chamber with alternating day/night temperatures of $25 \,^{\circ}\text{C}/20 \,^{\circ}\text{C}$ and a constant relative humidity (80%). Lighting treatments were kept throughout the experiment. A week later, half of the trays for each light treatment were irrigated with 20 mL per tray of a nutrient solution [20] once a week for 3 weeks, and the other half with a saline treatment, where 80 mM of NaCl were added to the nutrient solution. Microgreens were harvested and weighted when seedlings had four true leaves.

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2.2. Lighting Treatments

The experiment included three lighting treatments, namely a control (FL) provided by fluorescent lamps (Philips 36W/54-765) and two LED treatments, providing a red and blue spectrum (RB) and a red, blue and far-red spectrum (RB+FR), respectively (Figure 1). LED lamps used for the study were manufactured by Flytech s.r.l. (Belluno, Italy) and featured diodes with narrow bands in the Hyper Red (R, peak at 669 nm), Blue (B, peak at 465 nm) and Far-Red (FR, peak at 730 nm) spectral regions. The RB treatment featured a red:blue ratio of 3, as formerly suggested in a wide range of vegetables by Pennisi et al. [13,21] The RB+FR treatment additionally integrated a 15% far red (FR) radiation. Lamps used for the FL treatment featured an RB ratio of 0.74. Spectral properties were determined using an illuminance spectrophotometer (CL-500A, Konica Minolta, Chiyoda, Tokyo, Japan). In all treatments, a constant photosynthetic photon flux density (PPFD) of $150 \pm 5 \ \mu mol \ m^{-2} \ s^{-1}$ over the plant canopy was provided, and measured using a QSO (Apogee instruments, Logan, UT, USA) photosynthetic active radiation (PAR) Photon Flux Sensor (with equal sensitivity to red and blue radiation), connected with a ProCheck handheld reader (Decagon Devices Inc., Pullman, WA, USA) [13]. A photoperiod of 16 h light/8 h darkness was used in all the lighting treatments.





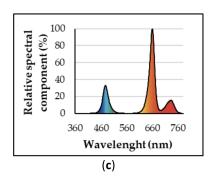


Figure 1. Spectral composition of the light supplied by FL (a), RB (b) and RB+FR (c) treatments used during the experiment.

2.3. Agronomical and Biochemical Parameters

Biomass production corresponding to the shoots weight (yield) was calculated at harvest and expressed as kg m $^{-2}$. Afterwards, ion content, total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), total carotenoid content (Car), total chlorophyll content (Chl) and fatty acids were analyzed.

Ion content was analyzed using 0.2 g of dried microgreens and quantified according to [4]. TPC, TFC, TAC, Chl and Car were determined using a methanolic extract, which was obtained from 50 mg of previously lyophilized leaves (Telstar Lyoquest 85 plus, Eco, Barcelona, Spain) in 1.5 mL of methanol. It was then vortexed and incubated overnight at 4 °C. After incubation, it was centrifuged at $16,000 \times g$ for 5 min at 4 °C. The supernatant was used as methanolic extract for subsequent analysis. TPC was determined by the Folin-Ciocalteu colorimetric method [22]. TFC was determined as described by Meda et al. [23]. TAC was evaluated in terms of their free radical-scavenging capacity [24]. Chl and Car were measured by extraction of 50 mg of sample in 1.5 mL of MeOH, and then absorbances at 652, 665 and 470 nm were measured in an UV-visible spectrophotometer (8453, Agilent, Santa Clara, CA, USA). The equations developed by Lichtenthaler and Buschmann [25] were used to determine the concentrations of chlorophyll a (Chl a = $16.72 \times A665 - 9.16 \times A652$), chlorophyll b (Chl b = $34.09 \times A652 - 15.28 \times A665$), Chl = Chl a + Chl b, Car = $(1000 \times A470 - 1.63 \times Chl a - 104.96 \times Chl b)/221$). Chl and Car were expressed as mg kg⁻¹ FW. Each of the three replicates was analyzed in triplicate. Fatty acid methyl esters (FAME) were identified and quantified following the methodology of O'Fallon et al. [26], with some modification [27].

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2.4. Statistical Analysis

Data were analyzed using Statgraphics Plus. An analysis of variance of agronomical and biochemical parameters (two-way ANOVA) was performed, in which the factors were lighting (FL, RB, RB+FR) and salinity (0 and 80 mM NaCl). When interactions were significant, they were included in the ANOVA; an LSD test was performed to compare lighting treatment and level of salinity.

3. Results and Discussion

3.1. Yield

Yield was affected by light and salinity (Table 1). It increased by 21% in both LED lighting treatments (RB and RB+FR) respect to FL. This finding is consistent with previous results on lettuce and basil, which demonstrated that a mixture of R and B diodes with an RB ratio of 3 resulted in higher yield compared to fluorescent light and other RB ratios [13,21]. This result may be explained by the fact that wavelengths of red and blue lights have been considered as the most convenient sources for enhancing vegetables growth, since they are absorbed by photosynthetic pigments more efficiently than other regions of the light spectrum [28,29]. In our experiment, the addition of FR to the spectrum did not result in a yield increase. However, Jin et al. [30] showed an increase in shoot weight when FR radiation was added to B+R radiation in lettuce 28 days after transplanting and Meng and Runkle [31] in lettuce and basil seedlings, a phenomenon that could be associated with faster leaf area expansion, which would ultimately result in increased light interception [30]. Possibly, in the current experiment, given that microgreens are harvested at early stage, the beneficial effects on leaf elongation induced by FR radiation were negligible, and therefore did not allow for increased light interception.

Table 1. Influence of light treatment and salinity (80 mM NaCl) on yield and nitrate content of purslane microgreens.

	Yield (kg m ^{−2})	Nitrate (mg kg^{-1} FW)
Light treatment (A)		
FL	1.47 ± 0.12 a	$6392 \pm 120 \text{ c}$
RB	$1.88 \pm 0.16 \mathrm{b}$	$1214\pm64~\mathrm{b}$
RB+FR	$1.87 \pm 0.14 \mathrm{b}$	556 ± 42 a
Salinity (B)		
0 mM NaCl	1.51 ± 0.09 a	$2857 \pm 501 \mathrm{b}$
80 mM NaCl	$1.97 \pm 0.15 \mathrm{b}$	$2585 \pm 529 \text{ a}$
Significant Differences		
A	*	***
В	**	**
$A \times B$	Ns ns	

Asterisk indicates significances at * p < 0.05, ** p < 0.01, *** p < 0.001; ns: non-significant. Different letters in the same column indicate significant differences. FL: fluorescent lamps, RB: LED Red+Blue, RB+FR: LED Red+Blue+Far-Red.

Salinity conditions increased microgreens yield by 23% (Table 1). It has been demonstrated that purslane can be considered as moderately salt tolerant, having the capacity of growing under salt stress conditions [6]. Accordingly, it was previously shown that purslane plants (cv. POR-2936) exposed to 100 mM NaCl presented higher shoot and root productivity, as compared to those grown with 0 mM NaCl [5]. However, the growth of an Aegean purslane accession was more suppressed under 140 mM NaCl than 70 mM NaCl [32], suggesting that the salinity concentration threshold to affect growth differs for each genotype [33]. Thus, increases in fresh weight rising salinity have been recorded in some purslane accessions (e.g., in Ac9), even up to 32 dS m⁻¹ [12]. On the other hand, Franco et al. [9] did not find yield differences when the EC in the nutrient solution was increased from 2.5 to 5.0 dS m⁻¹ when testing salinity response on purslane accession CM 01-215, while yield was instead reduced by 26% when salinity of 15.0 dS m⁻¹ was imposed.

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Finally, there was no interaction between light and salinity for yield (Table 1), in agreement with the results of Bantis et al. [34], who did not detect yield differences in spinach baby leaf among mildly saline (40 mM NaCl) and non-saline treatments for the different LEDs tested.

3.2. Anions and Cations

Nitrate content was affected by light and salinity (Table 1), reaching the highest values when microgreens were grown under FL light (6392 mg kg⁻¹ FW). This content was reduced by 81% and 91% when they were grown under RB and RB+FR, respectively, regarding FL light. The effects of light spectra on nitrate assimilation in plants are quite complex because the activity of nitrate reductase (NR) and its expression are affected by them. The high value of nitrate content found in microgreens grown under FL light could be due to the lamps used for this treatment featuring an RB ratio of 0.74, much lower than the others (RB ratio of 3). It has been recently demonstrated that red light is effective in reducing nitrate concentration in rocket by increasing NR activity [35], being mediated by phytochrome photoreceptors through transcriptional and posttranslational regulation [36]. However, the effects of blue light on nitrate uptake and utilization in plants seem to be much weaker than those of red light, although when it was combined with other light spectra, it showed positive effects by decreasing nitrate concentration [37]. Thus, Kyriacou et al. [19] demonstrated that combining red and blue light was more effective at promoting nitrate assimilation, resulting in lower nitrate residual concentrations in microgreens. Far-red light addition led us to a higher decrease in nitrate content respect to RB, in agreement with the results of Viršile et al. [38]. A possible explanation for this might be that FR light could induce the phosphorylation of NR through phytochrome-mediated signaling, which would result in its activation [39]. However, the involvement of NR in the response of purslane microgreens to FR and RB needs further investigation, since light effects on nitrate assimilation are plant species-specific [38].

The addition of NaCl reduced nitrate content by 9.5%. This result is consistent with that of Franco et al. [9], who demonstrated that when increasing salinity (>5.0 dS m $^{-1}$), the nitrate contents of purslane plants was decreased. The difference in nitrate accumulation in response to salinity is generally associated with the inhibition of NO_{3 $^-$} uptake by Cl $^-$ [40], which might occur by the interaction between these ions at the site of entrance and for ion transport [41,42]. However, a recent study in basil demonstrates that the interaction between nitrates and chlorines exerts a large effect on yield and metabolomics profile that cannot be satisfactorily explained only by an anion/anion antagonist outcome [43]. They suggest a dose-dependent effect, which is mainly due to the combination of a response to nutrient availability, and an inducible response to stress provoked when chloride concentration in the nutrient solution surpasses that which is accomplished to satisfy nutrient requirements.

There was a significant interaction between light and salinity for oxalate content. The highest content was obtained when microgreens were grown under RB light in non-saline conditions (5311 mg kg⁻¹ FW) and the lowest when they were grown under the same light in saline conditions (4375 mg kg⁻¹ FW); i.e., saline conditions provoked a reduction in oxalate by 17% (Figure 2). Furthermore, in non-saline conditions, the addition of FR light to RB provoked a significant decrease in oxalate content by 11%. Phytochrome mediates changes in the activities of many enzymes. Particularly, the activity of ascorbate oxidase was reported to increase by irradiation with continuous FR light [44,45], since ascorbic acid accumulation is controlled by the active phytochrome [46]. It has been proposed that oxalate is synthesized via three precursors—glyoxylate/glycolate, ascorbate and oxaloacetate [47]—ascorbic acid being a significant carbon source of oxalic acid in plants. Therefore, it may be advanced that the reduction in oxalate content in purslane microgreens growing under RB+FR lighting as compared with RB under non-saline conditions can be that FR light promotes the activity of ascorbate oxidase, reducing the concentration of ascorbic acid, a major substrate for synthesis of oxalic acid. To date, literature on the

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influence of light quality on oxalate content is limited. Qi et al. [48] applied four lighting treatments using FL lamps of different colors (red, blue, yellow and white) over a soilless crop of spinach, showing that oxalate content in spinach leaves under red light was 47.6% lower than that under white light. Gao et al. [49], in another study on hydroponic spinach using LED lamps with different ratio of red light to blue light (0.9, 1.2, and 2.2), concluded that treatment with more fraction of red light was favorable to reduce oxalate accumulation. These results, although elaborated with different crop species and light sources from the hereby presented experiment, substantiate the need for additional research on the role of light in oxalate content and ascorbic acid pathways with possible applications also for purslane.

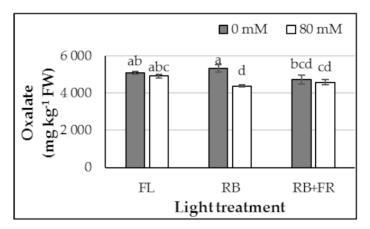


Figure 2. Effect of the different light (FL, RB and RB+FR) and salinity (0 and 80 mM NaCl) combinations on the oxalate content of purslane microgreens at harvest. Values are the mean \pm SE (n = 9). Different letters indicate significant differences ($p \le 0.05$).

The decrease in oxalate by adding NaCl to the nutrient solution was significant only when RB lighting was used. In previous studies, [50] suggested that when chloride or other anions are absorbed by plants, these anions compete for cations and depress oxalate synthesis. The decrease in oxalic acid accumulation on the leaves could therefore be associated to a competitive accumulation between oxalic acid with chloride ion, since purslane plant accumulates chloride ions in the leaves when submitted to saline stress [6]. Oxalate accumulation in vacuoles plays a vital function in balancing excess of inorganic cations over anions [33], regulating intracellular pH to neutralize excess cations [51]. Oxalate concentration was not affected by saline conditions in the other lighting treatments, which could be due to the influence of different spectra, which could affect the balance between cations and anions and/or the activity of ascorbate oxidase as previously mentioned in the case of FR lighting, although other mechanisms may operate; therefore, further research is needed.

There was a significant interaction between light and salinity for Cl, Na, Ca and K content. Cl and Na content had the same pattern among different treatments, with the highest contents being found when microgreens were grown under FL+NaCl treatment and the lowest under RB and RB+FR in non-saline conditions (Figure 3a,b). Accordingly, both in saline and non-saline conditions, Cl and Na contents of microgreens grown under LED lights were significantly reduced as compared to those grown under FL light. Therefore, spectral quality could modulate responses to a salinity stress probably through regulation of antioxidant enzymes and non-enzymatic systems, with phytochrome B1 playing a very important role in this process [52], but further research is needed for confirming this. As it was expected, the addition of NaCl to the nutrient solution significantly increased the Cl and Na content in each light treatment.

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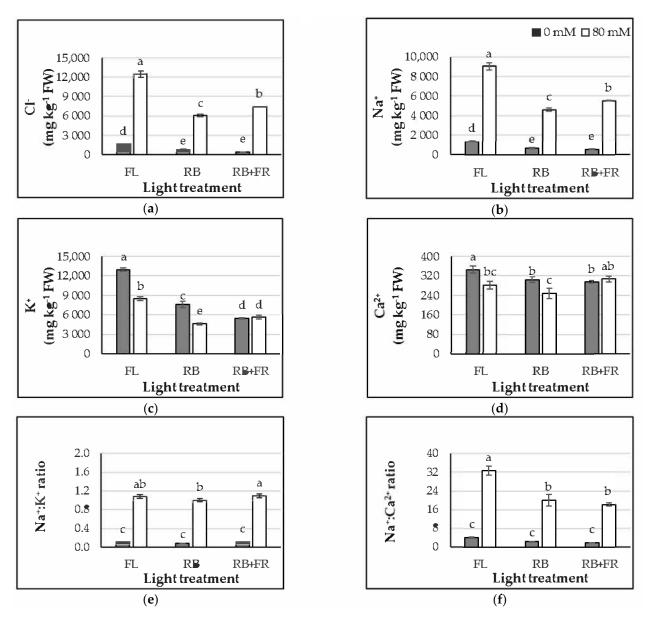


Figure 3. Effect of the different light (FL, RB and RB+FR) and salinity (0 and 80 mM NaCl) combinations on Cl⁻ (a), Na⁺ (b), K⁺ (c), and Ca²⁺ (d) content and Na⁺/K⁺(e) and Na⁺/Ca²⁺ (f) ratios of purslane microgreens at harvest. Values are the mean \pm SE (n = 9). Different letters indicate significant differences ($p \le 0.05$).

With respect to K content (Figure 3c), the lowest values (4601 mg kg⁻¹ FW) were found when the microgreens were grown under RB light in saline conditions, while the highest values (12,970 mg kg⁻¹ FW) were obtained when they were grown under FL light in non-saline conditions. Ca content had a similar pattern than K (Figure 3d), but there were no significant differences for lowest Ca and for the highest Ca values between RB and FL lights in saline and non-saline conditions, respectively. Furthermore, the addition of FR to the spectrum increased K and Ca contents in saline condition. However, salinity reduced their contents under RB and FL lights. The Na:K ratio was about 1 for saline conditions and about 0.1 for non-saline conditions, independently of the light treatment (Figure 3e). However, the Na:Ca ratio ranged between 32 for FL light and around 18 for LED lights in saline conditions, and between 4 and 2 for non-saline conditions for all lights (Figure 3f). Therefore, salinity could induce both osmotic stress and ionic toxicity associated with excessive Cl and Na uptake, leading to Ca and K shortage and to other nutrient imbalances [53], as was previously demonstrated in purslane [54]. Essentially, the K decrease in saline conditions can be explained by the antagonism of Na and K at

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uptake places in the roots, the effect of Na on K transport into the xylem or the inhibition of the uptake process [55]. Regarding the Ca decrease, it could be due not only to the accumulation of Na in the leaves, but also because of its reduced mobility and transport in plant due to the lower water uptake [56,57]. In summary, the addition of FR light led to a higher increase in Cl, Na, K and Ca content in leaves compared to RB light in saline conditions, maintaining the ratio Na/Ca and increasing the ratio Na/K.

3.3. Total Phenol Content, Total Flavonoids Content and Total Antioxidant Capacity

TPC was only affected by lighting, with the microgreens grown under the RB spectrum being those that reached the highest content (Table 2). Martínez-Zamora et al. [58] found that the TPC of carrot sprouts remained quite constant during a germination period of 7 d in darkness plus 10 d in a similar photoperiod in samples grown under RB LEDs, while Fl and RB+FR treatments reduced 24% and 12% the maximum TPC reached during growing, respectively. Purslane microgreens under RB+FR treatment showed the lowest TFC (around 14% lower than under FL and RB lights), while salinity reduced the TFC by 10%. In addition, the TAC was not affected by light or salinity treatments. However, Martínez-Zamora et al. [58] reported that under the above lighting conditions, carrot sprouts from the FL treatment reported 39.4% less TAC, by the same procedure used in this experiment, than those grown under RB LEDs. In fact, in a review concerning LED lighting as a key to modulate antioxidant compounds in plants, Loi et al. [59] stated that B, R and FR lights had shown to be able to increase the TPC and TFC in different plant commodities, and that the regulation of TPC and TFC by LEDs can be performed directly by inducing the expression of the key enzyme and indirectly by increasing the shikimic acid as precursor molecule [60]. Nevertheless, besides light intensity, their contents also depend upon plant species, cultivars and timing of LED exposure [59]. It is well established that salinity can enhance the synthesis of secondary metabolites in plants [61], producing reactive oxygen species, which are harmful to plant cells. Thus, polyphenols could help in improving TAC of plants at low to moderate salt concentrations [62]. Therefore, plants such as halophytes tolerant to stress could be interesting organisms for production of secondary metabolites, which would be useful for food and medicinal applications [63]. In our experiment, in contrast, TPC and TAC was not affected by salinity, and TFC was decreased when microgreens were grown with 80 mM NaCl (Table 2). However, in agreement with our results, He et al. [5] revealed that purslane grown with 0 and 100 mM NaCl had similar TPC, which decreased when plants were grown with 200 and 300 mM NaCl. According to the previous authors, TPC for purslane grown under higher salinity could be associated with their lower photosynthetic performance, although genetics and environment can be also affecting their accumulation [64]. It has also been demonstrated that purslane plants responded to NaCl stress by enhancing their antioxidative capacity and proline accumulation [32]. However, TAC is regulated by the level of stress associated to NaCl concentration. In our experiment, TAC was not affected by salinity, indicating an overtaking of antioxidant capacity face to salt stress, as could in the halophyte Enchylaena tomentosa at high salinity conditions [65]. Similar results were reported by Islam et al. [66], where low and medium levels of NaCl increased the TAC in wheat microgreens, whereas high saltconcentration (100 mM) suppressed the accumulation of antioxidants production, and by Valifard et al. [67], who showed that there was an increase and decrease in antioxidants in Salvia mirzayanii leaves because of mild and high salinity stress, respectively. Furthermore, in our experiment, salt stress decreased the TFC, in agreement with Islam et al. [66], who demonstrated that the production of an extract from wheat microgreen with above the concentration of 50 mM Na Cl resulted in a decrease in TFC, and with Zhou et al. [68], who showed that the TFC increased at low (25 mM) or moderate (50 mM) levels but declined at severe (75 and 100 mM) levels in Schizonepeta tenuifolia. It has been hypothesized that the biosynthesis of flavonoids can be encouraged by the changes in the cellular redox homeostasis, which are regulated by changing cellular redox potentials [69,70].

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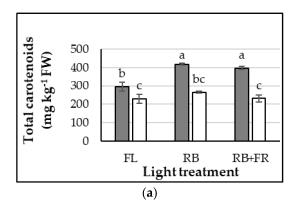
Table 2. Influence of light and salinity (80 mM NaCl) on the total phenolics, total flavonoids and antioxidant capa	icity of
purslane microgreens.	

	Total Phenolics (mg GA kg ⁻¹ FW)	Total Flavonoids (mg Rutin kg ⁻¹ FW)	Antioxidant Capacity (mg DPPH _{reduced} kg ⁻¹ FW)
Treatments (A)			
FL	$1176\pm21~\mathrm{b}$	$2829\pm72\mathrm{b}$	170 ± 3
RB	$1277\pm27~\mathrm{c}$	$2819 \pm 91 \text{ b}$	174 ± 5
RB+FR	931 ± 25 a	2417 ± 91 a	177 ± 6
Salinity (B)			
0 mM NaCl	1112 ± 64	$2834 \pm 99 \mathrm{b}$	175 ± 6
80 mM NaCl	1143 ± 69	2544 ± 98 a	172 ± 3
Significant Differences			
A	***	***	ns
В	ns	**	ns
$A \times B$	ns	ns	ns

Asterisk indicates significances at ** p < 0.01, *** p < 0.001, ns: non-significant. Different letters in the same column indicate significant differences. GA: gallic acid; DPPH: 2.2-diphenyl-1-picrythydrazyl. (FL: fluorescent lamps, RB: LED Red+Blue, RB+FR: LED Red+Blue+Far-Red).

3.4. Chlorophylls and Carotenoids

Regarding photosynthetic pigments (Car and Chl), a significant interaction between light and salinity factors was observed. The highest values for Car were obtained under LED lighting in non-saline conditions, whereas the lowest values were achieved in salinity conditions, without differences among factor combinations (Figure 4a). Regarding Chl, there were no differences between lighting treatments in non-saline conditions, while salinity conditions reduced their contents for every factor combination, reaching the lowest value under RB+FR treatment (Figure 4b). It has been demonstrated that light quantity and quality are the most important factors affecting chlorophyll and carotenoid contents because they are closely related to photosynthesis. In this context, blue LEDs, used alone or in combination with red light, increased the carotenoid and chlorophyll content [71,72]. Similarly, Samouliene et al. [73] found that higher concentrations of carotenoids and chlorophylls a and b were found when B light component was increased by 33% in comparison to lower B light dosages. Furthermore, Li and Kubota [71] demonstrated that supplemental FR decreased carotenoids and chlorophyll concentration by 11% and 14%. In our experiment, only Car was affected by light in non-saline conditions, increasing their concentrations under RB and RB+FR by 29% and 26%, respectively, in relation to FL light, possibly due to their higher percentage in B light of the two former lights. In contrast, adding FR to RB light did not affect either Car or Chl in non-saline conditions, but decreased Chl in saline conditions.



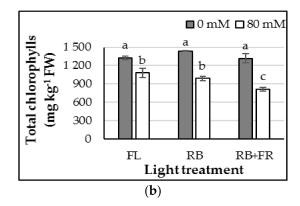


Figure 4. Effect of the different light (FL, RB and RB+FR) and salinity (0 and 80 mM NaCl) combinations on total carotenoids (a) and total chlorophylls (b) of purslane microgreens. Values are mean \pm SE (n = 3). Different letters indicate significant differences ($p \le 0.05$).

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Car and Chl in microgreens were reduced by salinity in the three lighting treatments. Camelle et al. [33] found a significant effect of the salinity, the nitrate to ammonium ratio and their interaction on Car and TChl accumulation in two purslane ecotypes, with salinity in plants growing with the 25:75 nitrate:ammonium ratio, leading to a drop in Car and Chl. A decrease in both parameters were also observed in the ecotype ET growing with the 66:33 ratio. He et al. [5] showed for total Car, that purslane plants grown with 100 mM NaCl reached the highest value followed by those grown with 0 mM, then 200 mM and later 300 mM NaCl. In the case of Chl, they did not find differences in Chl between purslane grown with 0 and with 100 mM NaCl, but they were higher than those grown with 200 and 300 mM NaCl. In addition, the decrease in the Chl of purslane plants with an increase in salinity in the nutrient solution has been previously demonstrated by Franco et al. [9], in agreement with our results. Finally, Zaman et al. [74] found that an increase in NaCl concentration from 0 to 200 mM caused a decrease in Chl in two genotypes of purslane. The results discussed here allow us to suppose that salinity stress could affect the photosynthetic performance in purslane due to salt accumulation in their leaves [75] and the decreases in Car and Chl, which can be altered according to level of salinity, the nitrate:ammonium ratio and the genotypes used.

3.5. Fatty Acids

The fatty acid content ranged between 303 and 418 mg 100 g^{-1} FW, without significant differences between treatments (Supplementary Table S1). The major fatty acid was α linolenic acid (LNA), whose proportion with respect to total fatty acids ranged between 54% and 60%, followed by linoleic acid (LA) (between 24% and 26%), palmitic acid (between 7% and 8%) and oleic acid (between 3% and 63%) (Figure 5). Smaller amounts of lauric acid, stearic acid, myristic acid, arachidic acid, behenic acid and lignoceric acid were also detected, although the latter was not detected in microgreens grown under RB+FR in both saline and non-saline conditions. As a consequence, oleic acid reached the highest content in these treatments. The LNA:LA ratio was significantly higher in the microgreens grown under FL in both saline and non-saline conditions, and under RB and RB+FR in non-saline conditions, compared with the ratio obtained under RB and RB+FR in saline conditions. To our knowledge, no study has been conducted on the effect of LED lighting on fatty acid content in superior plants, with most of the studies dealing with major of them having been conducted on microalgae. In these species, it has been demonstrated that the expression of fatty acid biosynthesis genes is regulated by different LEDs and that, consequently, different light intensities and wavelengths affect their fatty acid yields [76]. In our study, the change in fatty acid composition under RB+FR light increased the oleic acid content. In contrast, Sharma et al. [77] found that specifically polyunsaturated FAs were augmented, whereas monounsaturated FAs decreased in Phaeodactylum tricornutum grown under red light compared to white light. Future studies on the current topic are therefore recommended, using different spectra composition, in order to get a convenient fatty acid composition in purslane.

As regards to salinity, our results agree with those obtained by Carvalho et al. [10], who found that the total amount of fatty acids in purslane did not significantly change with increasing saline stress. Although the unsaturated fatty acids have emerged as general protectors against different abiotic and biotic stress [78], in our study, this effect was not observed, probably due to the salinity tolerance of purslane microgreens. However, Camalle et al. [33] observed differences with respect to total fatty acid content for the purslane ecotypes and nitrate to ammonium ratio tested, with an increase under saline conditions for ecotype ET compared to ecotype RN. In addition, changes in the fatty acid composition of leaves were detected and fatty acid contents were affected by salinity in borage, with LNA content being the most affected [79]. Therefore, besides salinity, differences in fatty acid content may be the result of differences in plant ecotypes or environmental and developmental factors, plant growth stage, nutrient solution composition, sample material or sampling procedures [33,80].

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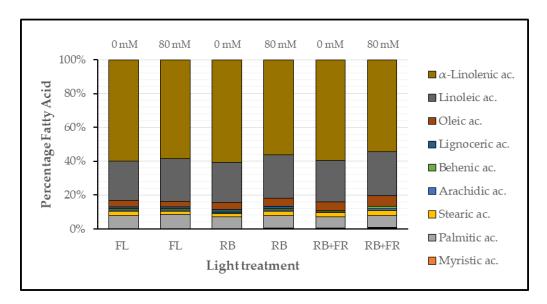


Figure 5. Percentage of fatty acid content of the aerial part of purslane microgreens for the different light treatment (FL, RB and RB+FR) and salinity (0 and 80 mM NaCl) combinations.

4. Conclusions

The results of this study indicate that it is convenient to grow purslane microgreens with 80 mM NaCl under RB and RB+FR lights, since these conditions improve yield and quality of the product, reducing the content of anti-nutritional compounds. In addition, the concentration of Cl and Na in the leaves can be reduced using the above conditions. However, a salinity of 80 mM reduced the content of total flavonoids, chlorophylls and carotenoids. Total fatty acids were not affected by salinity or lighting, but the specific composition of them can be modified by these factors. However, more research using different salinity conditions and spectra and intensity of LED lights is needed to target specific improvements of microgreens purslane quality. In further experiments, quality changes during shelf life should be elucidated.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10 .3390/horticulturae7070180/s1. Table S1: title, Fatty acids (mg gr $^{-1}$ FW) of purslane microgreens grown under the different light treatments (FL: fluorescent lamps, RB: LED Red Blue, RB+FR: LED Red Blue+Far-Red) and salinity (0, 80 mM NaCl) (n = 3).

Author Contributions: Conceptualization, M.d.C.M.-B. and J.A.F.; methodology, G.P., F.O. and A.C.; formal analysis, A.G.; investigation, A.G. and P.A.G.; resources, A.C. and J.A.F.; data curation, A.G. and F.O.; writing—original draft preparation, A.G., M.d.C.M.-B. and J.A.F.; writing—review and editing C.E.-G., F.A.-H., F.O., G.P., M.d.C.M.-B. and J.A.F.; supervision, F.O., C.E.-G., P.A.G. and F.A.-H.; funding acquisition, J.A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This project was financed by the Autonomous Community of the Region of Murcia no 20849/PI/18 through the grant call for projects for the development of scientific and technical research by competitive groups, included in the Regional Programme for the Promotion of Scientific and Technical Research (Action Plan 2018) of the Seneca Foundation-Science and Technology Agency of the Region of Murcia (Spain). The research also received funding from the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF), in the framework of the project "Light on Shelf Life" (CUP J56J20000410008).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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Acknowledgments: The elaboration of the manuscript was supported by a grant from the Fundación Séneca (reference 20555/IV/18, Call for Fellowships for Guest Researcher Stays at Universities and OPIS of the Region of Murcia) awarded to Francesco Orsini.

Conflicts of Interest: The authors declare no conflict of interest.

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