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Enhancing Growth and Glucosinolate Accumulation in Watercress (*Nasturtium officinale* L.) by Regulating Light Intensity and Photoperiod in Plant Factories

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Abstract: Recent advancements in light-emitting diode technology provide an opportunity to evaluate the correlation between different light sources and plant growth as well as their secondary metabolites. The aim of this study was to determine the optimal light intensity and photoperiod for increasing plant growth and glucosinolate concentration and content in watercress. Two-week-old seedlings were transplanted in a semi-deep flow technique system of a plant factory for 28 days under four photoperiod–light intensity treatments (12 h–266 μ mol·m⁻²·s⁻¹, 16 h–200 μ mol·m⁻²·s⁻¹, 20 h— $160 \text{ }\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, and 24 h— $133 \text{ }\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) with the same daily light integral. The mean values of shoot fresh and dry weights were the highest under the 20 h—160 μ mol·m⁻²·s⁻¹ treatment, although there was no significant difference. Net photosynthesis and stomatal conductance gradually decreased with decreasing light intensity and increasing photoperiod. However, total glucosinolate concentration was significantly higher under 20 h—160 μ mol·m⁻²·s⁻¹ and 24 h—133 μ mol·m⁻²·s⁻¹ compared with 12 h-266 µmol· m⁻²·s⁻¹ and 16 h-200 µmol· m⁻²·s⁻¹. The total glucosinolate content was the greatest under 20 h—160 µmol· m⁻²·s⁻¹ treatment. These data suggest that the 20 h—160 μ mol·m⁻²·s⁻¹ treatment promoted the maximum shoot biomass and glucosinolate content in watercress. This study supplies the optimal light strategies for the future industrial large-watercress cultivation.

Keywords: deep flow technique; glucosinolate; light-emitting diode; net photosynthesis; shoot biomass; watercress

1. Introduction

Watercress (*Nasturtium officinale* L.; Brassicaceae) is a semi-aquatic or aquatic perennial herb mainly cultivated in Asia, North and South America, and Europe [1]. Watercress is evaluated as an aquatic weed in some regions. It is used in soups (as garnish), fresh salads, and in other dishes [2]. The European Food Safety Authority has indicated that watercress is a safe vegetable of the group "herbs, edible flowers, and leaf vegetables" [3]. The US Centers for Disease Control and Prevention selected watercress as one of the crops containing the highest nutrient content per calorie [4]. It contains compounds such as vitamins, polyphenols, carotenoids, and isothiocyanates, and glucosinolates are the most crucial components present in watercress [2]. Watercress is a known medicine for treating cough, bronchial problems, and asthma [5]. Watercress has pharmacological actions such as antioxidant, anti-inflammatory, cardioprotective actions, antipsoriatic, antibacterial, and anticancer properties [1,2,6,7]. Because of the abundance of chemical components, watercress can be used in the food, medicine, and cosmetics industries.

Growing plants in a plant factory using artificial light is an efficient method of agricultural cultivation for combating climate change and worldwide food shortage [8]. Water shortages, unusual weather, and depletion of the agricultural land area result in a



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). decrease in field crop production globally [9,10]. Nevertheless, these environmental problems do not affect crop production in a closed plant factory as the conditions for growth are controlled using temperature regulation, air conditioning, air circulation fans, artificial light, nutrient solutions, and CO_2 enrichment [11,12]. Crops cultivated in a plant factory always depend on artificial light (light intensity, photoperiod, and light quality), which controls the photosynthetic process, plant physiology, biochemistry, and morphology [13,14]. Thus, upgrading the light source efficiency would significantly decrease the expense of the plant factory system, which in turn would promote sustainable cultivation because the impacts of ecological and costs could be decreased.

The lighting can be supplied for plants at uniform and fixed times with specificwavelength illumination in a plant factory because lighting schedules can be controlled, and particular photoperiods can be adjusted to promote plant growth and quality. For example, longer photoperiods increased the fresh weight of lettuce [15]. The growth of lettuce was increased with longer photoperiods and lower photosynthetic photon flux density (PPFD) at the same daily light integral (DLI) because the longer photoperiods compensated for a lower PPFD [16]. The growth of Achimenes cultivars grown under a low light intensity and longer photoperiods was higher as compared to that of those grown under a high light intensity and shorter photoperiods at the same DLI [17]. In addition, plant growth and morphology changes were reported due to changes in light intensity and photoperiod [18]. In general, these reports indicated that plant growth can be promoted under longer photoperiods with the same DLI. There have been several studies on the influences of different light intensities [19–21] and a combination of photoperiods and light intensities on plant biomass and secondary metabolites [15,22,23]. The optimum plant growth, yield, and quality can be obtained by controlling the light-emitting diode photoperiod and light quality [24,25]. Therefore, establishing a light regime that provides a favorable light photoperiod and intensity for plant growth and development is an essential step in cultivating plants in hydroponic systems in plant factories.

To date, several studies have been conducted on the influence of different light qualities, photoperiods, and light intensities on the growth and quality of watercress [26,27]. However, the effects of different light intensities in combination with different photoperiods on the growth and glucosinolate concentration and content of watercress grown in a plant factory have not yet been reported. For year-round production of good quality watercress in plant factories, it is important to understand the growth and quality responses to combined conditions of two light factors including photoperiod and light intensity. Thus, the aim of this study was to find the optimal light intensity and photoperiod treatment to increase plant growth and glucosinolate content in watercress. We hypothesized that plant growth and glucosinolate content in watercress with the increase of light intensity and photoperiod treatment.

2. Materials and Methods

2.1. Seedling Conditions

Watercress seeds were sown in rockwool cubes (240 holes; UR Rockwool, Suwon, Korea) and grown in a plant factory for 2 weeks. The air temperature and relative humidity in the plant factory were controlled at 20 ± 2 °C and $60 \pm 10\%$, respectively. White fluorescent lamps (TL5 14W/865 Philips, Amsterdam, Netherlands) were used for illumination. The photoperiod and PPFD were adjusted to 16 h per day and 150 µmol· m⁻²·s⁻¹, respectively. The Hoagland solution for watercress seedlings (electrical conductivity 0.8 dS·m⁻¹; pH 6.0) was supplied from 1 week after sowing.

2.2. Treatments

Two weeks after sowing, the seedlings were transplanted into four lighting treatments in a plant factory with the same daily light integral (11.52 mol m⁻² d⁻¹). Each treatment had 10 plants. The 12 h—266 µmol treatment photoperiod was set to 12 h per day with a PPFD of 266 µmol· m⁻²·s⁻¹. The 16 h—200 µmol treatment photoperiod was set to 16 h per day with a PPFD of 200 μ mol· m⁻²·s⁻¹. The 20 h—160 μ mol treatment photoperiod was set to 20 h per day with a PPFD of 160 μ mol· m⁻²·s⁻¹. The 24 h—133 μ mol treatment photoperiod was set to 24 h per day with a PPFD of 133 μ mol· m⁻²·s⁻¹. Plants were cultivated under a 7:3 ratio of red/blue light-emitting diodes (LEDs) for 28 days under 400 μ mol· mol⁻¹ CO₂ concentration and 60% \pm 10% relative humidity. The blue and red LEDs had a peak wavelength of 450 and 660 nm, respectively. The experiment was conducted with two replicates for each treatment. The Hoagland nutrient solution for watercress plants (EC 2.0 dS·m⁻¹; pH 6.0) was supplied for 28 days. The day and night air temperatures were controlled at 22 and 20 °C, respectively.

2.3. Measurement of Photosynthetic Parameters and SPAD Value

The net photosynthetic rate and stomatal conductance of fully expanded leaves were measured with a portable photosynthesis system (LI-6400; Li-Cor, Lincoln, NE, USA) at 28 days after transplantation. The measurement conditions in the leaf chamber, namely CO₂ concentration, leaf temperature, airflow rate, and PPFD, were maintained at 400 μ mol·mol⁻¹, 25 °C, 500 cm³·s⁻¹ and 500 μ mol·m⁻²·s⁻¹, respectively. The SPAD values were measured with a portable chlorophyll meter (502, Minolta Camera Co., Ltd., Tokyo, Japan). All parameters were recorded for 6 plants (*n* = 6) in each replication.

2.4. Measurement of Plant Growth Parameters

After 28 days of transplanting, the shoot fresh and dry weights and stem length were measured. The stem length and shoot fresh weight were determined using a ruler and an electronic scale (EW220-3NM, Kern & Sohn GmbH., Balingen, Germany), respectively. For determination of the shoot dry weight, samples were dried for one week in an oven (HB-502M; Hanback Sci, Suwon, Korea) at 70 °C and then weighed.

2.5. Determination of the Individual Glucosinolate Concentration in Watercress

The individual glucosinolate concentrations in the watercress were analyzed according to previous literature [28] but with some modifications. The shoots of watercress plants were collected at 28 days after transplanting, kept in a deep freezer at -70 °C after soaking in liquid nitrogen, then moved to a dry freezer (TFD5503, IL Shinbiobase Co., Ltd., Seoul, Korea) at -50 °C for 3 days and ground to powder. Glucosinolate was extracted in 70% (v/v) methanol with watercress powder (0.1 g) in a water bath for 5 min. Afterward, the samples were centrifuged at $12,000 \times g$ for 10 min, and the supernatant was analyzed as described by Lam et al. (2019) and Cuong et al. (2019) [28,29] to determine individual glucosinolate concentrations (glucobrassicin, 4-methoxyglucobrassicin, glucohirsutin, glucosiberin, and gluconasturtiin; Table 1). Desulfoglucosinolates were measured using a high-performance liquid chromatography (HPLC) system (1200 Infinity, Agilent Technologies, Santa Clara, CA, USA). An Inertsil ODS-3 (C18) column $150 \times 3.0 \text{ mm}^2$ i.d., particle size 3 µm (GL Science, Tokyo, Japan), was used with a column temperature of 40 °C, a wavelength of 227 nm, and a flow rate of 0.4 mL·min⁻¹. The individual glucosinolates were measured by response factors (ISO 9167-1, 1992) [30] and the HPLC peak area ratios and with reference to a desulfosinigrin external standard. Glucosinolate contents (µmol/plant DW) were presented as total glucosinolate concentration in the shoot $(\mu mol \cdot g^{-1} DW)$ multiplied by shoot dry weight (g).

2.6. Statistical Analysis

The growth and SPAD values were measured for six plants per replication. Photosynthetic parameters were measured for four plants per replication. The individual glucosinolate concentrations were measured for three plants per replication. For statistical analysis, one-way ANOVA was performed using SPSS 20.0 (SPSS, Inc., Chicago, IL, USA). Significant differences among treatments were verified at $p \le 0.05$, using Tukey's multiple range test.

Common Name	Side Chain Structure	Retention Time (min)	Response Factor
Glucobrassicin	indol-3-ylmethyl	17.16	0.29
4- Methoxyglucobrassicin	4-methoxyindol-3- ylmethyl	16.05	0.25
Glucohirsutin	8-methylsulfinyloctyl	13.78	1.1
Glucosiberin	7-methylsulfinylheptyl	16.77	1
Gluconasturtiin	2-phenylethyl	15.68	0.95

Table 1. Relative response factor values of the desulfoglucosinolates from watercress shoot extracts and their retention times on C18 column [28].

3. Results

3.1. Plant Growth Parameters, Chlorophyll Content, and Photosynthetic Parameters

The 12 h—266 µmol, 16 h—200 µmol, and 20 h—160 µmol treatments resulted in higher growth parameters relative to the 24 h—133 µmol treatment. The shoot fresh and dry weights were significantly higher (1.15 and 1.42 times, respectively) under the 20 h—160 µmol treatment compared to the 24 h—133 µmol treatment (Figure 1C,D). However, the stem length and SPAD value of the watercress were not significantly affected by photoperiod and light intensity combinations (Figure 1A,B).



Figure 1. Stem length (**A**), SPAD value (**B**), shoot fresh weight (**C**), and shoot dry weight (**D**) under different lighting treatments of 12 h—266 µmol· m⁻²·s⁻¹, 16 h—200 µmol· m⁻²·s⁻¹, 20 h—160 µmol· m⁻²·s⁻¹, and 24 h—133 µmol· m⁻²·s⁻¹. Different letters above bars show significant differences at $p \le 0.05$, using Tukey's multiple range test (n = 6).

The net photosynthesis and stomatal conductance were reduced with increasing photoperiod and decreasing light intensity (Figure 2). Specifically, the net photosynthesis

under 16 h—200 µmol, 20 h—160 µmol, and 24 h—133 µmol treatments was 1.38, 1.52, and 3.32 times lower than that of 12 h—266 µmol treatment in the study, respectively. The stomatal conductance under 16 h—200 µmol, 20 h—160 µmol, and 24 h—133 µmol treatments was 1.41, 1.92, and 2.08 times lower than that of 12 h—266 µmol treatment in this study, respectively (Figure 2). Moreover, the shoot fresh and dry weights of the watercress were significantly low under lower light intensity and longer photoperiod treatments (24 h—133 µmol) compared with 12 h—266 µmol, 16 h—200 µmol, and 20 h—160 µmol treatments. There was no significant difference in shoot fresh and dry weights among 12 h—266 µmol, 16 h—200 µmol, and 20 h—160 µmol treatments (Figure 1).



Figure 2. The net photosynthesis (**A**) and stomatal conductance (**B**) under different lighting treatments of 12 h— 266 µmol· m⁻²·s⁻¹, 16 h—200 µmol· m⁻²·s⁻¹, 20 h—160 µmol· m⁻²·s⁻¹, and 24 h—133 µmol· m⁻²·s⁻¹. Different letters above bars show significant differences at $p \le 0.05$, using Tukey's multiple range test (n = 4).

3.2. Total Glucosinolate Concentration and Content

These analyses indicate that the watercress contained five different desulfoglucosinolates (glucohirsutin, 4-methoxyglucobrassicin, glucoiberin, glucohirsutin, and gluconasturtiin). Among the five desulfoglucosinolates identified, gluconasturtiin presented the highest concentration (Table 2). Gluconasturtiin accumulation in the shoot increased under 24 h—133 µmol treatment and had the highest concentration (82.51% of the total glucosinolate concentration). However, there was no significant difference in gluconasturtiin concentration among 24 h—133 µmol, 16 h—200 µmol, and 20 h—160 µmol treatments or between 20 h—160 µmol and 12 h—266 µmol treatments. The highest glucosiberin, 4-methoxyglucobrassicin, and glucohirsutin concentrations (13.61%, 3.79%, and 2.39% of the total glucosinolates, respectively) were observed under 20 h—160 µmol treatment. There was no significant difference in glucosiberin concentration among 24 h—133 µmol, 16 h—200 µmol, and 12 h—266 µmol treatments. There was no significant difference in glucohirsutin concentration among four treatments (24 h—133 µmol, 16 h—200 μmol, 12 h—266 μmol, and 20 h—160 μmol). There was no significant difference in 4-methoxyglucobrassicin concentration among 24 h—133 µmol, 16 h—200 µmol, and 20 h—160 µmol treatments or among 24 h—133 µmol, 16 h—200 µmol, and 12 h—266 µmol treatments. The highest glucobrassicin concentration (8.80% of the total glucosinolate) was recorded under 24 h—133 µmol treatment. There was no significant difference in glucobrassicin concentration among 24 h-133 µmol, 16 h-200 µmol, and 12 h-266 µmol treatments (Table 2). Overall, the total glucosinolate concentration was the greatest at 24 h—133 µmol treatment and was 1.28-fold higher than that of the 12 h—266 µmol treatment. There were no significant differences in total glucosinolate concentration in shoots between the 12 h and 16 h treatments or 20 h and 24 h treatments (Figure 3A). However, the total glucosinolate content in the shoot was the highest under 20 h—160 µmol treatment because glucosinolate contents (µmol/plant DW) were presented as total glucosinolate concentration in the shoot (μ mol g⁻¹ DW) multiplied by shoot dry weight (g). There were

no significant differences in total glucosinolate content in shoot among 24 h—133 μ mol, 16 h—200 μ mol, and 12 h—266 μ mol treatments (Figure 3B).

Table 2. The individual glucosinolate concentration of watercress under different lighting treatments of 12 h—266 μ mol· m⁻²·s⁻¹, 16 h—200 μ mol· m⁻²·s⁻¹, 20 h—160 μ mol· m⁻²·s⁻¹, and 24 h—133 μ mol· m⁻²·s⁻¹.

Lighting Treatment	Individual Glucosinolate Concentration in Watercress Shoots (mg \cdot g ⁻¹ DW) ^z				
	Siber	Hirsu	Brassi	Metho	Nastur
12 h—266 µmol	1.04b	0.53	1.83ab	0.58b	15.69b
16 h—200 μmol	0.63b	0.45	1.75ab	0.68ab	18.92a
20 h—160 µmol	3.36a	0.59	1.40b	0.94a	18.81ab
24 h—133 µmol	0.46b	0.53	2.21a	0.79ab	20.71a
Significance ^y	***	NS	**	***	*

^z Siber: glucosiberin, Hirsu: glucohirsutin, Brassi: glucobrassicin, Metho: 4-methoxyglucobrassicin, Nastur: gluconasturtiin. ^y Different letters show a significant difference within each treatment according to Tukey's multiple range test at Not Significant (NS) (p > 0.05), * $p \le 0.05$; ** p < 0.01; and *** p < 0.001 (n = 3).



Figure 3. The glucosinolate concentration (**A**) and content (**B**) in watercress shoots under different lighting treatments of 12 h—266 µmol· m⁻²·s⁻¹, 16 h—200 µmol· m⁻²·s⁻¹, 20 h—160 µmol· m⁻²·s⁻¹, and 24 h—133 µmol· m⁻²·s⁻¹. Different letters above bars show significant differences at $p \le 0.05$, using Tukey's multiple range test (n = 3).

4. Discussion

4.1. Plant Growth Parameters, Chlorophyll Content, and Photosynthetic Parameters

Previous reports have indicated that higher light intensities enhanced growth and promoted crop production [31,32]. This was possible because of the wider expansion of the leaf under the higher light intensity treatment. A larger leaf leads to more light interception, which might have resulted in a significant increment in the shoot fresh and dry weights under higher light intensity [31]. The growth of ice plants under a fluorescent lamp, red LEDs, and blue LEDs was significantly higher under a higher light intensity treatment (150 µmol· m⁻²·s⁻¹) than under a lower light intensity treatment (120 µmol· m⁻²·s⁻¹) [20]. The biomass, stem diameter, and root/shoot ratio of soybean were higher under 400 and 500 µmol· m⁻²·s⁻¹ than under 100 µmol· m⁻²·s⁻¹ [33]. The leaf area was reduced by shade conditions [34]. Similarly, plant dry matter production of soybean decreased with decreasing light intensity [35]. Leaf fresh weight of *Arabidopsis thaliana* was significantly higher under a higher light intensity as compared to that of plants grown under a low light intensity at 6 weeks after transplanting [36]. Moreover, the fresh weight of watercress subjected to a long day (16 h) was significantly higher than that subjected to a short day (8 h) [26]. The fresh and dry weights of quinoa increased under a short photoperiod and

high light intensity treatment [37]. Likewise, the biomass of the watercress increased under high light intensity and short photoperiod treatments.

Reductions in light intensity may influence the carbon balance in the plant. Rates of physiological process increase, while the photosynthetic yield decreases [33]. Normally, it is expected that the shading conditions or lower light intensities restrict leaf growth and result in smaller leaf areas with thinner leaves, reduced chlorophyll content, and thinner palisade tissues, leading to lower light-harvesting [38,39]. Furthermore, there is a reduction in stomatal conductance and density, which leads to poor CO₂ transportation under low light conditions. The electron transition from PSII to PSI is obstructed, whereas the activity and number of enzymes that participate in the Calvin cycle undergo a change. All of this results in a reduced carbon dioxide assimilation rate and a reduced net photosynthetic rate under low light conditions [33]. Previous reports have indicated that the main biochemical restraint related to shadow-associated down-adjustment of net photosynthetic rate is a decrease in the activity or amount of rubisco [33,40]. Photosynthetic capacity was reduced under low light conditions because carbon was restricted [41]. For example, low light intensity reduced the photosynthesis rate in pak choi [42] and soybean [33]. Thus, the net photosynthesis and stomatal conductance were reduced with increasing photoperiod and decreasing light intensity.

4.2. Total Glucosinolate Concentration and Content

Glucosinolates are bioactive compounds typically found in cruciferous group plants. It has been indicated that long days could enhance glucosinolate accumulation in Arabidopsis [43] and watercress [26]. Low light intensity increased the concentrations of 4-methoxyglucobrassicin, glucobrassicin, and neoglucobrassicin in pak choi [44]. The antioxidant activity and the total phenolic content of Orthosiphon stamineus under an open environment were higher than shade-grown conditions [45]. Total phenolic content in the leaves of *Ipomoea batatas* was higher under 16 than 8 h at a light intensity of 150 μ mol·m⁻²·s⁻¹ [46]. Antioxidant capacity and total phenolic content in lettuce continuously increased with increasing photoperiods in 150 μ mol·m⁻²·s⁻¹ conditions. Specifically, the phenolic content in lettuce was highest at 24 h under 150 μ mol· m⁻²·s⁻¹ and was 5.3-fold higher than under a 12 h period treatment [47]. Likewise, in the results of this experiment, total glucosinolate concentration was significantly higher under 20 h—160 µmol and 24 h—133 µmol compared with 12 h—266 μ mol and 16 h—200 μ mol. This indicates that the long photoperiods had a more photomorphogenic effect than a photosynthetic one. However, the total glucosinolate concentration was not significantly different between the 20 and 24 h photoperiods. The results indicate that the total glucosinolate concentration could increase with increasing photoperiods under low light intensity. However, it might reach a saturation point under low light intensity and long photoperiod. Expanding the photoperiod in weak light intensity conditions has a slight compensatory effect because it can decrease the negative influences of the weak light stress.

5. Conclusions

The results indicated that a photoperiod of 20 h at 160 μ mol·⁻²·s⁻¹ enhanced total glucosinolate content and plant biomass of watercress grown in a plant factory. Further studies can investigate the influence of light quality from LEDs on the productivity and bioactive compounds of watercress grown in a plant factory. Moreover, the results also suggested that longer photoperiod induction was a potential method for watercress glucosinolate production. There is great potential to apply these results to improve the quality of watercress plants and enhance the efficiency in watercress cultivation in plant factories.

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