

Research Article

Light-Emitting Diode Light Quality Influences Germination and Sprout Characteristics of Motherwort

Woo-Hyun Lee, Mewuleddeg Zebro, and Jae-Yun Heo 💿

Department of Plant Science, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea

Correspondence should be addressed to Jae-Yun Heo; jyheo@gwnu.ac.kr

Received 19 June 2023; Revised 9 September 2023; Accepted 19 September 2023; Published 27 September 2023

Academic Editor: Timothy Omara

Copyright © 2023 Woo-Hyun Lee et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Motherwort (*Leonurus japonicus* Houtt) is an important medicinal plant known for its excellent antioxidant properties. Nonetheless, in South Korea, its availability remains limited due to the challenge of low seed germination rates, affecting field production. To tackle this problem, it is imperative to focus on controlled production methods. In our study, we conducted experiments in a laboratory setting, employing various combinations of light-emitting diode (LED) lights to cultivate motherwort. The influence of LED light quality on seed germination, initial growth, and functionality of motherwort is evaluated. The germination rate of motherwort ranged from $36.1 \pm 7.35\%$ to $75.0 \pm 1.60\%$, and the highest value was observed in the red LED 100% treatment. The mean shoot length also varied depending on LED light quality. The longest shoot length (3.45 ± 0.13 g) was obtained in the red LED 100% treatment. The highest shoot weight (0.266 ± 0.011 cm) and root weight (0.051 ± 0.008 cm) were obtained in the red LED 70% and blue LED 30% mixed treatment. The total phenolic content of the motherwort sprout ranged from 2.50 ± 0.30 mg GAE/g to 3.01 ± 0.09 mg GAE/g, with the highest value from the white LED (control) treatment, but no significant differences were observed among the treatments. The red LED 30% and blue LED 70% mixed treatment showed the highest total flavonoid content (11.62 ± 0.79 mg QE/g) and DPPH radical scavenging activity ($57.64 \pm 2.95\%$). The red LED 70% and blue LED 30% mixed treatment had a similar level of DPPH scavenging activity as the control, and there was no positive or negative effect on the functionality of motherwort sprout. Overall, the results suggest that the red LED 70% and blue LED 30% mixed treatment can be effectively used to increase productivity in motherwort without decreasing its important quality.

1. Introduction

Motherwort (*Leonurus japonicus* Houtt) is a biennial plant belonging to the Lamiaceae family and is widely distributed in East Asia, including South Korea, China, and Japan. Motherwort grows to a length of 1 to 1.5 meters and produces square stems. This versatile herb has played a crucial role in the ethnobotanical practices of diverse communities worldwide. In traditional Chinese medicine, it is highly esteemed for its calming attributes, with its aerial components, including leaves and stems, expertly brewed into herbal teas or tinctures to alleviate anxiety, bolster cardiovascular health, and address menstrual discomfort [1–3]. Furthermore, European herbalism taps into motherwort's aerial parts to support female reproductive health and mitigate heart-related diseases [4]. Previous research has demonstrated that motherwort exhibits a significantly elevated polyphenol content in comparison to other indigenous plants in Korea [5]. This indicates that motherwort exhibits excellent antioxidant activity, leading to continuous research for the development of medicines and the production of functional foods [6, 7]. Moreover, there is growing consumer interest in edible medicinal plants with high antioxidant activity in the Korean agricultural market, and ongoing efforts are being made to utilize wild plant species like motherwort as a vegetable sprout [8].

Understanding germination and sprout characteristics in plants holds paramount importance, as these processes are foundational to a plant's life cycle, growth, and overall health. Germination marks the inception of a plant's life, awakening dormant seeds and initiating root and shoot growth [9]. Comprehending germination dynamics allows for the optimization of seedling establishment, ensuring a robust start for crop plants or native species in ecological restoration efforts [10]. In addition, sprout characteristics, including shoot elongation, branching, and leaf development, significantly impact a plant's ability to harness light energy and compete for resources [11]. These attributes directly influence photosynthetic capacity, biomass accumulation, and reproductive success, all of which hold farreaching implications for agricultural productivity, ecosystem dynamics, and global food security [12].

However, in South Korea, wild seeds with low seed germination rates and frequent nonuniform characteristics are harvested and used for cultivation. As a result, the production of motherwort in the field is low, and a significant proportion is imported to meet domestic demand since Korean production is insufficient [13]. Despite these problems, research on improving the productivity of motherwort is lacking. To address this issue, it is essential to explore the potential of light-emitting diodes (LEDs) as a solution. LEDs have gained prominence as a cutting-edge lighting technology in agriculture and horticulture, offering distinct advantages over traditional lighting sources, chiefly due to their precise spectral control, enabling growers to tailor light quality to specific plant requirements [14]. This precise control enhances photosynthetic efficiency, reduces energy consumption, and allows for optimized growth conditions, ultimately leading to improved crop yields and resource-efficient cultivation practices [15].

Similar problems have been reported in *Gloxinia*, and it has been observed that yield increases when optimized LED wavelengths are applied in controlled environments [16]. However, it should be noted that the effectiveness of LED application varies depending on LED quality in plants [17, 18]. To confirm the potential of LED application in effectively addressing productivity issues in motherwort, it is necessary to examine the actual effects of LED treatments on the plants. Therefore, this study aims to track the seed germination, sprout growth characteristics, and functionality of motherwort following the application of various LED wavelengths. The objectives of this study are as follows: (1) to assess whether LED application can contribute to improving the productivity and quality of motherwort and (2) to establish fundamental insights regarding the appropriate wavelengths for motherwort production should the effects of LED prove beneficial.

2. Materials and Methods

2.1. Plant Material and LED Source. This study was conducted using seeds of motherwort produced by Danong (Namyangju, Korea) in 2022 at the Plant Breeding Laboratory of the Department of Plant Science, Gangneung–Wonju National University. The 36 seeds were sown in 10 litre pots filled with horticultural soil media. The seeds employed for this experiment were carefully chosen through visual inspection to exclude any visibly damaged or irregular seeds. Three pots $(13 \text{ cm} \times 17 \text{ cm})$ were prepared for each treatment. Throughout the establishment and development of the seedlings, the temperature and relative humidity were maintained at $23 \pm 1^{\circ}$ C and $60 \pm 10\%$, respectively, with a 12/12 h (light/dark) photoperiod. In this experiment, we utilized a total of five LED light sources. These sources consisted of two single LED sources and three mixed LED sources, obtained from Bissol LED (Seoul, Korea). The single LED light treatments consisted of 100% red (R100) LEDs emitting light at a wavelength of 650 nm and 100% blue (B100) LEDs emitting light at a wavelength of 450 nm. The mixed LED light treatments consisted of the following ratios: red 70%: blue 30% (R70: B30), red 50%: blue 50% (R50: B50), and red 30% : blue 70% (R30 : B30). The control group was treated with white LEDs. The distance between the cultivation bed and the light source was 30 cm, and the position of each cultivation tray was changed every three days to reduce the uneven distribution of light resulting from plant position (Figure 1).

2.2. Evaluation of Seed Germination Characteristics and α -Amylase Activity. The number of germinated seeds was counted as individuals whose rootlets had elongated by 1 mm or more each day [19], from the first day to 19 days after seed sowing. These data were used to assess important seed germination characteristics, including the germination rate (GR), germination energy (GE), and the median germination time (T_{50}). The germination rate (GR) (%) was calculated by dividing the number of germinated seeds by the total number of seeds over the experimental period, as described by Kim et al. [20]. Germination energy (GE) (%) was determined as the percentage of germinated seeds on the 9th day. The T_{50} value, which represents the median germination time, was calculated using the equation proposed by Coolbear et al. [21] and can be described as follows:

$$T_{50}$$
 (days) = Ti + (Tj - Ti) × $\frac{(N/2 - Ni)}{(Nj - Ni)}$. (1)

Here, *N* represents the final number of germinated seeds, while Ni and Nj denote the total number of seeds germinated on consecutive counts at times Ti and Tj, respectively, where Ni < N/2 < Nj.

0.5 g of seedling leaves from motherwort were harvested from each treatment, with three replications, and ground in a mortar and pestle with liquid nitrogen. The resulting powder was homogenized with 4 mL of Tris-HCl extraction buffer (0.02 M Tris-HCl, pH 6.5). The homogenate was then transferred to a 15 mL Falcon tube and centrifuged at 12,000 rpm for 30 minutes at 4°C. The clear supernatant was carefully collected in a new tube and stored at -20°C for further analysis. The determination of α -amylase activity was performed spectrophotometrically using the dinitrosalicylic acid (DNS) method. The assay mixture consisted of 1% starch, 1 mL of enzyme extract, color reagent (consisting of 5.3 M potassium sodium tartrate, 96 mM DNS, and ultrapure water), and additional ultrapure water. To initiate the reaction, 1 mL of the starch solution was transferred to a 15 mL tube and equilibrated at 20°C for 3 minutes. Subsequently, 1 mL of the enzyme extract was added to the solution and incubated for 3 minutes. After that, 1 mL of the color reagent was added to terminate the reaction, and the mixture was placed in a boiling water bath for 15 minutes. The solution was then cooled on ice at room temperature, and 9 mL of ultrapure water was added to dilute the reaction mixture. The absorbance of the resulting solution was measured at 540 nm using a spectrophotometer, and the concentration of α -amylase was determined using maltose as the standard. The enzyme activity was calculated using the following equation:

Enzyme activity
$$\left(\frac{\text{unit}}{\text{mg solid}}\right) = \frac{(\text{unit/mL enzyme})}{(\text{mg solid/mL enzyme})},$$
(2)

where mg solid represents the final concentration of starch in 2 mL of the reaction solution, which is 0.5%.

2.3. Evaluation of Growth Characteristics and Functionality in Motherwort Sprout. To evaluate the growth characteristics of motherwort sprouts, the following parameters were measured 20 days after seed sowing, using six plants per replication with three replications: shoot length, root length, fresh weight of shoots and roots, number of leaves, leaf area, and functionality. The total shoot and root lengths were measured using a ruler, and their fresh weights were determined using an electronic balance (WBA-220; Witeg Labortechnik GmbH, Wertheim, Germany).

The functionality of motherwort was evaluated with three replications by measuring the total polyphenol content, total flavonoid content, and DPPH radical scavenging ability. Sample extraction for analysis was conducted using a slightly modified method based on Lee et al. [22]. First, 0.05 g of powdered aerial parts of motherwort, which were dried at 60°C for 3 days and ground, were placed in a 15 mL tube containing 3.5 mL of 100% methyl alcohol. The mixture was sonicated using an ultrasonic bath (Elmasonic S30, Elma Schmidbauer GmbH, Germany) for 30 minutes. After sonication, the tube was centrifuged at 4°C and 4500 rpm for 20 minutes. Next, 3.5 mL of the supernatant, excluding the precipitate, was collected as the extracted solution. The same extraction process was repeated with 1.5 mL of solution to ensure consistency among the treatment groups. Finally, to standardize the concentration across all treatment groups, the solution was adjusted to a total volume of 5 mL for each treatment group.

The total phenol content was determined using a slightly modified method described by Geleta et al. [23]. For the assay, $500 \,\mu$ L of motherwort extract was mixed with 2,500 μ L of 10% Folin–Ciocalteu reagent and incubated for 3 minutes. Then, 20% sodium bicarbonate was added to the mixture, followed by incubation for 1 hour at room temperature. The absorbance of the resulting solution was measured at 765 nm using a microplate reader. A calibration curve was created using a standard material prepared by dissolving 0.5% gallic acid in 10 mL of ethanol and mixing it with 1,550 μ L of distilled water. The total polyphenol content was calculated as gallic acid equivalent per gram of dry weight (mg GAE/g) using the calibration curve.

The flavonoid content was measured using Chang's method [24]. In this method, $100 \,\mu$ L of 10% AlCl₃, $1.5 \,\text{mL}$ of methanol, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water were added to $500 \,\mu$ L of motherwort extract. The mixture was stirred for 1 minute and incubated in the dark for 30 minutes. Then, the absorbance was measured at 415 nm using a microplate reader. The total flavonoid content was calculated as quercetin equivalent per gram of dry weight (mg QE/g) based on the calibration curve.

The DPPH radical scavenging activity was tested based on the method described by Brand-Williams et al. [25]. In this method, $100 \,\mu$ L of the extraction solution was added to a 0.1 mM DPPH solution diluted with methanol. The mixture was mixed well for 3 minutes using a stirrer and then incubated in the dark for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) =
$$\left[\frac{(\text{Abscontrol} - \text{Abssample})}{\text{Abscontrol}}\right] \times 100,$$
 (3)

where Abscontrol is the absorbance value of the control (without the sample) and Abssample is the absorbance value of the tested sample.

2.4. Statistical Analysis. For data comparison, the analysis of variance (ANOVA) was conducted using SPSS software (Version 28; IBM, New York, USA). Following the ANOVA, the Duncan test was performed at a significance level of $P \le 0.05$ to compare the means and determine significant differences between the groups.

3. Results and Discussion

3.1. Seed Germination Characteristics. The effect of LED light quality on seed germination characteristics in motherwort is shown in Table 1. Seed germination generally started on the seventh day of the experiment and was completed by the 18th day regardless of treatments (Figure 2). The seed germination rate of motherwort ranged from $36.11 \pm 7.35\%$ to $75.00 \pm 1.60\%$ (Table 1). A high germination rate was observed in LED conditions such as R100 and R70:B30, compared to the control, while the other LED combinations

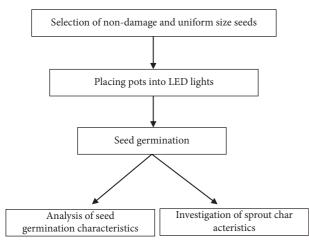


FIGURE 1: Experimental procedure flowchart.

TABLE 1: Effect of LED quality on seed germination characteristics of motherwort.

Treatment	Germination rate (%) ^z	Germination energy (%)	T_{50}
R100	71.30 ± 3.34^{ab}	63.89 ± 4.24^{a}	$7.22 \pm 0.05^{\circ}$
R70:B30	75.00 ± 1.60^{a}	54.63 ± 1.85^{ab}	7.47 ± 0.05^{bc}
R50:B50	59.26 ± 11.38^{abc}	44.44 ± 6.99^{b}	7.18 ± 0.14^{c}
R30: B70	50.00 ± 3.21^{bc}	$19.44 \pm 2.78^{\circ}$	$8.18\pm0.20^{\rm a}$
B100	$36.11 \pm 7.35^{\circ}$	$22.22 \pm 6.99^{\circ}$	7.82 ± 0.29^{ab}
White	63.89 ± 10.52^{ab}	$40.74\pm9.12^{\rm b}$	7.63 ± 0.11^{bc}

^zWithin each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p \le 0.05$). ±indicates standard error.

showed a lower level of germination rate (Table 1). Among the treatment groups used in this experiment, the groups with the highest and lowest germination rates were R70:B30 and B100, respectively. Germination energy showed a significantly higher value compared to the control group in the treatment group containing 50% or more of the RED wavelength, while a lower value was found in the treatment group with a high blue light ratio. T_{50} ranged from 7.18 ± 0.14 to 8.18 ± 0.20 days and showed a low value in the treatment group containing 50% or more of the RED wavelength, similar to germination energy (Table 1).

Seed germination rate is an important indicator as it directly affects the potential yield and profitability for farmers. Germination energy and T_{50} also provide valuable insights into the seed's ability to germinate and establish itself uniformly under specific environmental conditions. It is generally desirable to have a higher germination rate and germination energy, as well as a lower T_{50} , indicating efficient and uniform germination [26]. It is worth noting that R100 and R70: B30 conditions were more effective in improving seed germination characteristics in motherwort, as indicated by their higher germination rates, germination energy, and lower T_{50} . Furthermore, it is noteworthy that the α -amylase activity was significantly higher in R100 and R70: B30 compared to other LED conditions (Figure 3), which could be another factor influencing the improved seed germination characteristics in these treatments.

During seed germination, various physiological, biochemical, and molecular changes occur [27]. One essential step is the conversion of stored starch into sugars, which serve as an energy source for the developing embryo [28]. This conversion is facilitated by an enzyme called α -amylase, which breaks down starch into smaller glucose molecules through hydrolysis. Research has shown that seeds with higher α -amylase activity during germination are more efficient at breaking down starch [29], resulting in increased availability of glucose for the growing embryo. This enhanced energy availability could accelerate the germination process and lead to better seedling establishment. On the other hand, seeds with lower α -amylase activity may cause delays or difficulties in starch breakdown and impede seedling development.

Previous study has reported that the activity of α -amylase in certain plants can be influenced by LED light wavelengths [30]. In our study, we also observed that red light promotes the activation of α -amylase during seed germination of motherwort. Red light stimulates phytochromes, light-sensitive pigments involved in seed germination [31]. When seeds are exposed to red light, phytochrome molecules in their inactive Pr form convert to the active Pfr form. This conversion initiates a signaling cascade, including changes in gene expression, ultimately leading to increased production of α -amylase [32]. Interestingly, our findings indicate that the R70:B30 light combination is the most effective in promoting seed germination and α -amylase activity in motherwort. Blue light, sensed by cryptochromes, can affect α -amylase activity negatively, and a higher proportion of blue light inhibits seed germination in some plants compared to a higher proportion of red light [33]. However, it should be noted that blue light plays a role in regulating various physiological and developmental processes, such as the synthesis of photoreceptors and growth hormones [34]. The distinct roles of red and blue light suggest that a balanced proportion of these wavelengths could create a more efficient signaling network and meet the physiological needs of the plant during germination, compared to using only red light. This could result in improved seed germination. While further research is necessary to determine the exact reasons behind the promotion of motherwort seed germination under a higher proportion of red light, our results strongly indicate that the proportion of red and blue light is crucial for the seed germination characteristics of motherwort. A higher proportion of red light definitely provides an advantage during motherwort seed germination definitely.

3.2. Growth Characteristics of Motherwort Sprout. The results of examining the growth characteristics of motherwort sprouts under different light-quality treatments are shown in Table 2. The shoot length ranged from 0.933 ± 0.047 to 3.458 ± 0.135 cm, with the highest plant height observed in R100 and the lowest in B100. The number of leaves was above 6.000 in all treatments except R100, which had 5.166 ± 0.205 . The leaf area was highest in the control at 5.818 ± 0.113 cm² and lowest in B100 at 3.115 ± 0.206 cm²,

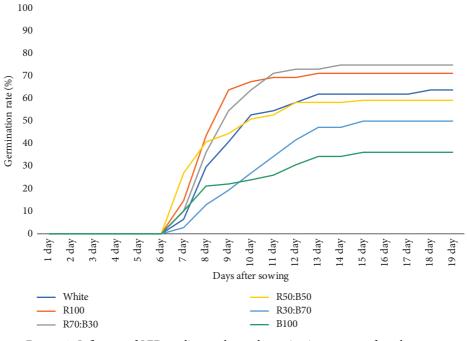


FIGURE 2: Influence of LED quality on the seed germination pattern of motherwort.

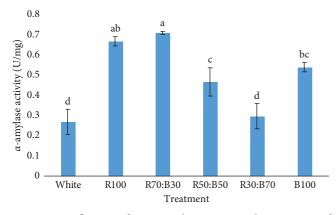


FIGURE 3: Influence of LED quality on α -amylase activity of motherwort. Different letters are significantly different according to Duncan's multiple range test ($p \le 0.05$). Error bar indicates the standard error of the mean.

which also had the shortest shoot length. The root length was highest in R70: B30 at 9.983 ± 0.348 cm and lowest in R100 and B100 at 6.683 ± 0.393 cm. The fresh shoot weight was heaviest in R70: B30 at 0.266 ± 0.011 g, significantly larger than in R100 at 0.113 ± 0.008 g. The fresh root weight was heaviest in R50: B50 at 0.058 ± 0.007 g and lightest in R100 at 0.006 ± 0.004 g. In sum, a significant difference ($p \le 0.05$) was observed in total fresh weight among treatments, and R70: B30 had the highest weight. In this regard, R70: B30 was found to be effective for increasing productivity when considering total weight including germination rate. It has been reported that red light can promote photosynthesis and gibberellin synthesis, resulting in increased shoot growth or biomass production [35]. Hence, this result is consistent with the previous result. Interestingly, a mixed treatment with a higher proportion of red LED and a lower proportion of blue LED is better for promoting the growth of motherwort sprouts than a single red LED treatment. Although blue light can inhibit shoot elongation [12], it is known to have a role in promoting stomatal opening [36], which can increase CO_2 uptake. For these reasons, it is assumed that the resulting light spectrum is ideal for promoting both photosynthesis and growth in motherwort sprouts when these two wavelengths of light are combined in an R70: B30 ratio.

In this experiment, the total phenolic compound content of the motherwort sprout ranged from 2.50 ± 0.30 to $3.01 \pm 0.09 \text{ mg} \cdot \text{GAE/g}$ (Table 3). The highest value was observed in the control, but no significant differences were detected among the treatments. Regarding the total flavonoid content of the motherwort sprout, the highest value was found at a statistically significant level ($p \le 0.05$) among the treatment groups at $11.62 \pm 0.79 \text{ mg} \cdot \text{QE/g}$ in R30: B70, and the second-highest value was observed at 9.16 ± 0.52 mg·QE/g in the control group (Table 3). The rest of the treatment groups were found to contain total flavonoid content in the range of 4.68 ± 0.71 to 6.03 ± 0.49 mg QE/g, and it was confirmed that they were overall lower than the two treatments. The DPPH scavenging activity was the highest at $57.64 \pm 2.95\%$ in R30: B70, which had the highest flavonoid content, and the lowest at $34.04 \pm 0.64\%$ in R100, which had the lowest content (Table 3). Among the remaining treatments, it was confirmed that the control and R70:B30 showed the highest DPPH radical scavenging activity after R30: B70.

The results obtained from this study have shown that LED light does not significantly affect the accumulation of total phenolic content in motherwort sprout. Le et al. [37] have reported an increase in total phenolic content with LED light exposure. Park et al. [38] have found no effect or even

TABLE 2: Effect of LED quality on growth characteristics of motherwort.

Treatment	Shoot length	Number of	Leaf area	Root length	Fresh weight (g)		
freatment	(cm) ^z	leaf	(cm^2)	(cm)	Shoot	Root	Total
R100	3.458 ± 0.135^{a}	6.000 ± 0.000^{a}	4.702 ± 0.130^{b}	$6.683 \pm 0.300^{ m b}$	0.240 ± 0.008^{ab}	$0.020 \pm 0.002^{\mathrm{bc}}$	$0.260 \pm 0.010^{ m b}$
R70:B30	$1.308 \pm 0.054^{ m b}$	6.166 ± 0.115^{a}	5.470 ± 0.180^{a}	9.983 ± 0.348^{a}	0.266 ± 0.011^{a}	0.051 ± 0.008^{a}	0.317 ± 0.018^{a}
R50:B50	$0.958 \pm 0.054^{\circ}$	$6.000 \pm 0.000^{\mathrm{a}}$	4.866 ± 0.132^{b}	9.066 ± 0.373^{a}	0.230 ± 0.009^{b}	$0.058 \pm 0.007^{\mathrm{a}}$	0.288 ± 0.015^{ab}
R30: B70	$1.000 \pm 0.061^{\circ}$	$6.000 \pm 0.000^{\mathrm{a}}$	$4.128 \pm 0.156^{\circ}$	7.366 ± 0.416^{b}	$0.156 \pm 0.010^{\circ}$	0.010 ± 0.002^{c}	$0.166 \pm 0.012^{\circ}$
B100	$0.933 \pm 0.047^{\circ}$	5.166 ± 0.205^{b}	3.115 ± 0.206^{d}	$6.683 \pm 0.393^{ m b}$	0.113 ± 0.008^{d}	$0.006 \pm 0.004^{\circ}$	0.119 ± 0.008^{d}
White	1.250 ± 0.086^{b}	6.000 ± 0.000^{a}	5.818 ± 0.113^{a}	$7.250 \pm 0.487^{\mathrm{b}}$	$0.235 \pm 0.008^{\mathrm{b}}$	$0.032 \pm 0.005^{\mathrm{b}}$	0.267 ± 0.012^{b}

^zWithin each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p \le 0.05$). \pm indicates standard error.

TABLE 3: Effect of LED quality on functionality of motherwort.

Treatment	Total phenol content (mg·GAE/g) ^z	Total flavonoid content (mg·QE/g)	DPPH radical scavenging activity (%)
R100	2.90 ± 0.06^{a}	$4.68 \pm 0.71^{\circ}$	$34.04 \pm 0.64^{\circ}$
R70:B30	2.64 ± 0.30^{a}	$6.03 \pm 0.49^{\circ}$	$50.98 \pm 1.54^{\mathrm{ab}}$
R50:B50	2.50 ± 0.30^{a}	$5.05 \pm 0.26^{\circ}$	47.33 ± 2.59^{b}
R30: B70	2.73 ± 0.21^{a}	11.62 ± 0.79^{a}	57.64 ± 2.95^{a}
B100	2.67 ± 0.06^{a}	$5.68 \pm 0.46^{\circ}$	$49.28 \pm 2.20^{ m b}$
White	3.01 ± 0.09^{a}	$9.16 \pm 0.52^{ m b}$	51.69 ± 2.91^{ab}

^zWithin each column, means followed by different letters are significantly different according to Duncan's multiple range test ($p \le 0.05$). ±indicates standard error.

a decrease in total phenolic content. These results suggest that the response to accumulating phenolic content in plants by LED treatment is species specific, and the effect of LED light on total phenolic content in motherwort sprout is limited. One of significant discovery from this study is that the total flavonoid content increases significantly when there is a red-to-blue light ratio of 30:70. It has been reported that flavonoid biosynthesis is regulated by different classes of photoreceptors in plants, including phytochromes and cryptochromes [39]. These photoreceptors sensitively respond to different wavelengths of light and have distinct roles in regulating flavonoid biosynthesis [40]. Blue light can stimulate the activity of cryptochromes, which are involved in regulating the flavonoid biosynthesis pathway, while red light can enhance the expression of genes involved in this pathway in motherwort sprout [41]. Together, these two wavelengths of light work synergistically to promote the accumulation of total flavonoid content. Based on previous results, it is assumed that a 30:70 red-to-blue light ratio could provide the optimal balance of wavelengths for maximizing flavonoid accumulation in different plant species [42, 43]. This previously conducted study is in line with the current investigation. In contrast, higher ratios of red light may lead to a much suppression of cryptochrome activity, which could result in lower flavonoid accumulation [44]. Similarly, higher ratios of red light may not provide enough blue light to promote optimal gene expression for flavonoid biosynthesis in motherwort sprout.

Although total phenolic and flavonoid content are definitely important indicators of antioxidant activity, DPPH radical scavenging activity is considered more important because it directly measures the ability of a substance to scavenge free radicals, which can cause cellular damage and contribute to various diseases [45]. A wide range of antioxidants can improve DPPH radical scavenging activity, including vitamin C, carotenoids, flavonoids, and phenolic compounds [46]. The important fact is that both red and blue light are known to activate the production of various antioxidants in plants. In this experiment, a higher level of total phenolic or flavonoid content does not guarantee a higher level of DPPH radical scavenging activity because we only observed the total phenolic and flavonoid content. Therefore, the results indicate that different levels of DPPH radical scavenging activity might result from the accumulation of other kinds of antioxidants. Apart from this fact, it was found that the application of R30: B70 could be appropriate in terms of functionality based on DPPH radical scavenging activity. In addition, it has been found that R70: B30 does not improve functionality in motherwort sprout compared to the control, but it does not have a lower level of functionality. This result strongly suggests that R70: B30 is good for the improvement of productivity in motherwort without a decrease in important traits that are crucial for the optimal functionality and productivity of the motherwort plant.

4. Conclusion

Nowadays, diverse wild plant species are actively introduced for functional food production and fresh consumption, but most of them suffer from low production ability due to their poor seed germination quality. In this study, we verified that LED quality is an important factor in improving productivity in motherwort, and LED quality can affect the functionality of motherwort. Notably, LED conditions such as R100 and R70:B30 proved effective in improving seed germination rates and efficiency, crucial for producers aiming to enhance productivity. In addition, the study identifies the balanced red and blue light combination of R70:B30 as particularly advantageous, promoting both growth and productivity. Our results can promote better utilization of motherwort in the food industry in Korea and can be used as an important example to develop strategies to enhance the productivity and quality of wild plant species.

Data Availability

The datasets used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Woo-Hyun Lee carried out the experiment, performed the statistical analysis, and wrote a draft manuscript. Mewuleddeg Zebro performed the experiment and contributed to revise manuscript. Jae-Yun Heo coordinated the study, supervised the experiment, and contributed to the writing of the manuscript. All authors have read and given their approval for the final manuscript to be submitted.

References

- J. Wang, Y. Mao, Y. Ma et al., "Diterpene synthases from *Leonurus japonicus* elucidate epoxy-bridge formation of spiro-labdane diterpenoids," *Plant Physiology*, vol. 189, no. 1, pp. 99–111, 2022.
- [2] Y. Xu, H. Yang, J. Chi, F. Wang, and H. Bao, "Simultaneous determination of eight components in *Leonuri Herba* from different habitats by HPLC-DAD," *Journal of Chromato*graphic Science, vol. 58, no. 5, pp. 464–470, 2020.
- [3] L. L. Miao, Q. M. Zhou, C. Peng, Z. H. Liu, and L. Xiong, "Leonurus japonicus (Chinese motherwort), an excellent traditional medicine for obstetrical and gynecological diseases," A comprehensive overview," Biomedicine & Pharmacotherapy, vol. 117, Article ID 109060, 2019.
- [4] R. H. Zhang, Z. K. Liu, D. S. Yang, X. J. Zhang, H. D. Sun, and W. L. Xiao, "Phytochemistry and pharmacology of the genus *Leonurus*: the herb to benefit the mothers and more," *Phytochemistry*, vol. 147, pp. 167–183, 2018.
- [5] E. J. Kim, J. Y. Choi, M. R. Yu et al., "Total polyphenols, total flavonoid contents, and antioxidant activity of Korean natural and medicinal plants," *Korean Journal of Food Science and Technology*, vol. 44, no. 3, pp. 337–342, 2012.
- [6] E. Y. Kang, H. K. Kim, J. Y. Jung et al., "Combined extract of *Leonurus Japonicus Houtt, Eclipta prostrata* L., and *Pueraria lobata* Ohwi improved hot flashes and depression in an ovariectomized rat model of menopause," *Foods*, vol. 10, no. 1, p. 180, 2021.
- [7] M. N. Park, E. S. Um, M. A. Rahman et al., "Leonurus japonicus Houttuyn induces reactive oxygen species-mediated apoptosis via regulation of miR-19a-3p/PTEN/PI3K/AKT in U937 and THP-1 cells," Journal of Ethnopharmacology, vol. 291, pp. 115129–129, 2022.
- [8] S. N. Jang, G. O. Lee, S. Lee, J. G. Yun, H. S. Shin, and K. H. Son, "Effect of super absorbent polymer on germination and growth of safflower and amaranth sprouts," *Journal of Bio-Environment Control*, vol. 30, no. 1, pp. 37–45, 2021.

- [9] A. S. Ali and A. A. Elozeiri, "Metabolic processes during seed germination," Advances in seed biology, pp. 141–166, 2017.
- [10] A. Gardarin, F. Coste, M. H. Wagner, and C. Dürr, "How do seed and seedling traits influence germination and emergence parameters in crop species?" *Seed Science Research*, vol. 26, no. 4, pp. 317–331, 2016.
- [11] K. Spaninks, J. Van Lieshout, W. Van Ieperen, and R. Offringa, "Regulation of early plant development by red and blue light: a comparative analysis between *Arabidopsis thaliana* and *Solanum lycopersicum*," *Frontiers in Plant Science*, vol. 11, Article ID 599982, 2020.
- [12] R. Paradiso and S. Proietti, "Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: the state of the art and the opportunities of modern LED systems," *Journal of Plant Growth Regulation*, vol. 41, no. 2, pp. 742–780, 2022.
- [13] M. W. Han, J. S. Park, Y. S. Kwak et al., "Studies on quality control of domestic *Leonurus japonicus* Houttuyn," *Korean Journal of Medicinal Crop Science*, vol. 24, no. 6, pp. 451–457, 2016.
- [14] A. Viršilė, M. Olle, and P. Duchovskis, "LED lighting in horticulture. Light emitting diodes for agriculture: smart lighting," in *Light Emitting Diodes for Agriculture*, S. Dutta Gupta, Ed., Springer, Singapore, pp. 113–147, 2017.
- [15] M. Al Murad, K. Razi, B. R. Jeong, P. M. A. Samy, and S. Muneer, "Light emitting diodes (LEDs) as agricultural lighting: impact and its potential on improving physiology, flowering, and secondary metabolites of crops," *Sustainability*, vol. 13, no. 4, p. 1985, 2021.
- [16] S. Sudthai and S. Sakhonwasee, "Reproductive development, seed yield and seed quality of gloxinia (*Sinningia speciosa*) in a closed plant production system as influenced by LED light quality and intensity," *The Horticulture Journal*, vol. 91, no. 1, pp. 24–32, 2022.
- [17] A. Gimenez, M. D. C. Martínez-Ballesta, C. Egea-Gilabert et al., "Combined effect of salinity and LED lights on the yield and quality of purslane (*Portulaca oleracea* L.) microgreens," *Horticulturae*, vol. 7, p. 180, 2021.
- [18] S. Zhen, P. Kusuma, B. Bugbee, T. Kozai, G. Niu, and J. Masabni, "Toward an optimal spectrum for photosynthesis and plant morphology in LED-based crop cultivation," in *Plant Factory Basics, Applications and Advances*, Academic Press, San Diego, CA, USA, 2022.
- [19] D. H. Lee, S. H. Kim, B. Tolera, and J. Y. Heo, "Effect of seed priming on the germination properties of *Allium hookeri*," *International Journal of Horticultural Science and Technology*, vol. 10, pp. 203–210, 2023.
- [20] S. H. Kim, D. C. Jang, M. Zebro, and J. Y. Heo, "Effects of various combinations of red and blue LEDs on seed germination and growth in Indian spinach (*Basella alba L.*)," *Italus Hortus*, vol. 29, no. 1, pp. 196–205, 2022.
- [21] P. Coolbear, A. Francis, and D. Grierson, "The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds," *Journal of Experimental Botany*, vol. 35, no. 11, pp. 1609–1617, 1984.
- [22] S. Y. Lee and J. Y. Heo, "Combined treatment with gibberellic acid and thidiazuron improves fruit quality of 'Red Dream' grape cultivar," *Notulae Scientia Biologicae*, vol. 15, no. 2, Article ID 11499, 2023.
- [23] B. T. Geleta, J. C. Lee, and J. Y. Heo, "Antioxidant activity and mineral content in unripe fruits of 10 apple cultivars growing in the northern part of Korea," *Horticulturae*, vol. 9, 2023.

- [24] C. C. Chang, M. H. Yang, H. M. Wen, and J. C. Chern, "Estimation of total flavonoid content in propolis by two complementary colorimetric methods," *Journal of Food and Drug Analysis*, vol. 10, pp. 178–182, 2002.
- [25] W. Brand-williams, M. E. Cuvelier, and C. L. W. T. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT-Food Science & Technology*, vol. 28, no. 1, pp. 25–30, 1995.
- [26] S. Anese, E. A. A. da Silva, A. C. Davide et al., "Seed priming improves endosperm weakening, germination, and subsequent seedling development of *Solanum lycocarpum*," *Seed Science & Technology*, vol. 39, no. 1, pp. 125–139, 2011.
- [27] S. Liu, W. Wang, H. Lu, Q. Shu, Y. Zhang, and Q. Chen, "New perspectives on physiological, biochemical and bioactive components during germination of edible seeds: a review," *Trends in Food Science & Technology*, vol. 123, pp. 187–197, 2022.
- [28] J. C. de Carvalho, J. F. Gonçalves, A. V. Fernandes et al., "Reserve mobilization and the role of primary metabolites during the germination and initial seedling growth of rubber tree genotypes," *Acta Physiologiae Plantarum*, vol. 44, no. 8, 2022.
- [29] H. Kaur, N. Gupta, and G. Kaur Gill, "Sole and combined effect of micronutrients on germination and seedling growth attributes of sweet corn (*Zea mays L. saccharata*)," *Journal of Plant Nutrition*, vol. 46, no. 10, pp. 2473–2487, 2023.
- [30] N. K. Nemahunguni, S. Gupta, M. G. Kulkarni, J. F. Finnie, and J. Van staden, "The effect of biostimulants and light wavelengths on the physiology of *Cleome gynandra* seeds," *Plant Growth Regulation*, vol. 90, no. 3, pp. 467–474, 2020.
- [31] M. M. Neff, "Light-mediated seed germination: connecting phytochrome B to gibberellic acid," *Developmental Cell*, vol. 22, no. 4, pp. 687-688, 2012.
- [32] D. Panda, S. Mohanty, S. Das et al., "The role of phytochromemediated gibberellic acid signaling in the modulation of seed germination under low light stress in rice (O. sativa L.)," *Physiology and Molecular Biology of Plants*, vol. 28, no. 3, pp. 585–605, 2022.
- [33] G. Wang, Y. Chen, H. Fan, and P. Huang, "Effects of lightemitting diode (LED) red and blue light on the growth and photosynthetic characteristics of *Momordica charantia* L," *Journal of Agricultural Chemistry and Environment*, vol. 10, no. 1, pp. 1–15, 2021.
- [34] J. Ni, Y. Liao, M. Zhang et al., "Blue light simultaneously induces peel anthocyanin biosynthesis and flesh carotenoid/ sucrose biosynthesis in mango fruit," *Journal of Agricultural* and Food Chemistry, vol. 70, no. 50, pp. 16021–16035, 2022.
- [35] S. Tripathi, Q. T. N. Hoang, Y. J. Han, J. I. Kim, and J. I. Kim, "Regulation of photomorphogenic development by plant phytochromes," *International Journal of Molecular Sciences*, vol. 20, no. 24, pp. 6165–65, 2019.
- [36] S. Vialet-chabrand, J. S. A. Matthews, and T. Lawson, "Light, power, action! interaction of respiratory energy-and blue light-induced stomatal movements," *New Phytologist*, vol. 231, no. 6, pp. 2231–2246, 2021.
- [37] A. T. Le, J. K. Yu, G. D. Han, T. K. Do, and Y. S. Chung, "Potential use of colored LED lights to increase the production of bioactive metabolites *Hedyotis corymbosa Lam*," *Plants*, vol. 11, no. 2, p. 225, 2022.
- [38] S. Y. Park, J. H. Bae, M. M. Oh, and M. M. Oh, "Manipulating light quality to promote shoot growth and bioactive compound biosynthesis of *Crepidiastrum denticulatum* (Houtt) Pak & Kawano cultivated in plant factories," *Journal of*

Applied Research on Medicinal and Aromatic Plants, vol. 16, Article ID 100237, 2020.

- [39] Y. Cheng, H. Chen, Y. Zhao, X. Cheng, L. Wang, and X. Guo, "Effect of light quality on polyphenol biosynthesis in three varieties of mung bean sprouts with different color seed coats," *Plant Cell Reports*, vol. 42, no. 2, pp. 253–268, 2023.
- [40] E. V. Kharshiing, O. I. L. Mawphlang, V. Lama, R. Bhattacharjee, and L. Sahoo, "Manipulation of light environment for optimising photoreceptor activity towards enhancing plant traits of agronomic and horticultural importance in crops," *The Journal of Horticultural Science and Biotechnology*, vol. 97, no. 5, pp. 535–551, 2022.
- [41] C. Lin, "Blue light receptors and signal transduction," *The Plant Cell*, vol. 14, no. suppl 1, pp. S207–S225, 2002.
- [42] J. W. Song, S. R. Bhandari, Y. K. Shin, and J. G. Lee, "The Influence of red and blue light ratios on growth performance, secondary metabolites, and antioxidant activities of *Centella* asiatica (L.) Urban," *Horticulturae*, vol. 8, no. 7, p. 601, 2022.
- [43] T. G. Nam, Y. J. Lim, and S. H. Eom, "Flavonoid accumulation in common buckwheat (*Fagopyrum esculentum*) sprout tissues in response to light," *Horticulture, Environment, and Biotechnology*, vol. 59, no. 1, pp. 19–27, 2018.
- [44] D. Okello, Y. Chung, H. Kim et al., "Antioxidant activity, polyphenolic content, and FT-NIR analysis of different Aspilia africana medicinal plant tissues," Evidence-based Complementary and Alternative Medicine, vol. 2021, Article ID 9917810, 11 pages, 2021.
- [45] O. A. Ojo, F. L. Oyetayo, A. S. Oladipo, and V. O. Oluwatosin, "Polyphenolic contents, free radical scavenging properties, and enzyme inhibitory activities of Acacia nilotica (L.) delile seed and pod extracts," *Vegetos*, vol. 10, 2023.
- [46] A. Zeb, S. Khan, and S. Ercisli, "Characterization of carotenoids, chlorophylls, total phenolic compounds, and antioxidant activity of *Brassica oleracea* L var. *botrytis* leaves from Pakistan," *Biologia*, vol. 77, no. 2, pp. 315–324, 2022.