JEE ISSN: 2288-1220
https://doi.org/10.5141/jee.22.021
Journal of Ecology and Environment (2022) 46:16

RESEARCH

The effects of LED light quality on ecophysiological and growth responses of *Epilobium hirsutum* L., a Korean endangered plant, in a smart farm facility

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ARTICLE INFO
Received March 17, 2022
Revised June 23, 2022
Accepted June 28, 2022
Published on July 11, 2022

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Background: *Epilobium hirsutum* L. is designated as an endangered plant in South Korea located in Asia, due to the destruction of its habitats through the development of wetlands. Therefore, in this study, in order to find a light condition suitable for the growth and ecophysiological responses of *Epilobium hirsutum* L., those of this plant under treatment with various light qualities in a smart farm were measured.
Results: In order to examine the changes in the physiological and growth responses of *Epilobium hirsutum* L. according to the light qualities, the treatment with light qualities of the smart farm was carried out using the red light: blue light irradiation time ratios of 1:1, 1:1/2, and 1:1/5 and a red light: blue light: white light irradiation time ratio of 1:1:1. As a result, the ecophysiological responses (difference between leaf temperature and atmospheric temperature, transpiration rate, net photosynthetic rate, intercellular CO_2 partial pressure, photosynthetic quantum efficiency) to light qualities appeared differently according to the treatments with light qualities. The increase in the blue light ratio increased the difference between the leaf temperature and the atmospheric temperature and the photosynthetic quantum efficiency and decreased the transpiration rate and the intercellular CO_2 partial pressure. On the other hand, the white light treatment increased the transpiration rate and intercellular CO_2 partial pressure and decreased the temperature difference between the leaf temperature and the ambient temperature and photosynthetic quantum efficiency.
Conclusions: The light condition suitable for the propagation by the stolons, which are the propagules of *Epilobium hirsutum* L., in the smart farm, is red, blue and white mixed light with high net photosynthetic rates and low difference between leaf temperature and atmospheric temperature.

Keywords: artificial light, light stress, photosynthesis, rhizome, stolon, vegetative propagation, wetland plant

Introduction

Nowadays, one of the most important issues related to ecology in the world is the restoration of endangered species, and the cause of the threat is largely due to man-made habitat destruction (Primack 2004). The loss of these species can also adversely affect the ecosystem as endangered species mainly are specialist that play an important role in inter-species interaction within the ecosystem (Dehling et al. 2021). In order to maintain the population sizes of endangered species due to habitat destruction, approaches should be attempted from a restoration ecological viewpoint. Ecological restoration refers to the attempt to return an ecosystem to a certain point in time in the past, but it is difficult or impossible. Therefore, ecological restoration should proceed through realistic goal setting (Falk et al. 2006).

As an ecological restoration method as such, a method of reintroducing the endangered species into their natural habitats can be used (Clements 2013). However, in order to re-introduce a certain species and lead to the resettlement of the species, a problem that a certain number of the individual plant species should be secured in advance should be solved. In addition, in the case of reintroduction, the degree to which the genetic diversity of the population that will be introduced is similar to that of the original population is one of the issues that must be considered (Falk et al. 2006). Furthermore, species preservation through reintro-

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duction is not always successful (Allen 1994). Therefore, rather than collecting and introducing a small number of the endangered plant from another area, a measure to secure a large number of individuals with genetic diversity close to that of the individuals of the original population through artificial cultivation and proliferation including vegetative propagation in a facility where the effects of the surrounding environments are minimized is necessary.

However, for propagation studies as such, first, basic ecophysiological research on the plant should precede. Ecophysiological approaches to the restoration of rare plants can improve the outcome of restoration so that the plants can settle and grow in disturbed environments by analyzing the interactions between the plants and the environments (Valliere et al. 2021). The smart farm is an optimal technology for such research. A smart farm is a facility that can improve plant productivity by monitoring the physical environment, soil, fertilizer, water supply, and plant growth based on information communication technologies (ICT), the Internet of things (IoT), and big data analysis (Jayaraman et al. 2016).

A smart farm can use sunlight and artificial light as light sources for plant cultivation (Mahdavian and Wattanapongsakorn 2017). However, using only sunlight as a light source can be economical for crop cultivation (Mahdavian and Wattanapongsakorn 2017), but it is not suitable for studies on the optimal light quality for plant cultivation. Therefore, using light-emitting diode (LED) light as a light source for such studies is appropriate. Whereas fluorescent lamps, incandescent lamps, high pressure sodium lamps, high pressure mercury lamps, and metal halides, which are light sources used as artificial light sources, have wide spectrums instead of single wavelengths and cannot supply a sufficient amount of photosynthetic photon flux density (PPFD) so that it is difficult to expect high plant productivity, LED light enables selecting certain optical wavelengths, has a long lifespan, and reaches the maximum output immediately after the switch is turned on so that high light frequencies can be set to save power (Dutta Gupta and Agarwal 2017; D'Souza et al. 2017). In addition, since it generates less heat than other light sources, heat damage to plants can be reduced, so that the distance between the light source and the plant can be maximally reduced in cultivation to use the cultivation space efficiently (Dutta Gupta and Agarwal 2017).

Epilobium hirsutum L. is a C3 plant that inhabits mainly humid areas of the Northern Hemisphere (Shamsi and Whitehead 1974; Arens et al. 2000). In the Himalayas, *Epilobium hirsutum* L. is a mountain plant that occurs mainly between 1,000 and 2,750 m, and in Northern Europe, Norway, and Sweden, it is distributed up to 600 m (Shamsi and Whitehead 1974). In Scandinavia, *Epilobium hirsutum* L. inhabits the low alpine belts on the south-facing slopes (Shamsi and Whitehead 1974). The areas where *Epilobium hirsutum* L. inhabits are limitedly distributed in areas where water supply is smooth, such as coastal areas, creek dikes and ditches, moist woodlands, and wetlands (Shamsi and Whitehead 1974).

Epilobium hirsutum L. grows upright up to about at least 1 m, and the leaves on the stem are in narrow oval shapes (Chung et al. 2017). The stem is thickly developed, and the base is lignified (Shamsi and Whitehead 1974). The stolons of *Epilobium hirsutum* L. spread out around the plant to form adventitious roots, which lead the stolons into the underground to develop into rhizomes (Shamsi and Whitehead 1974). The stolons spread on the soil surface develop rosettes at the tip of the stems, the aerial part withers to death after autumn, and the rhizomes develop the aerial part again the following year (Shamsi and Whitehead 1974). There are phellogens inside the rhizomes so that aerenchymas can be developed (Batten 1918). In Asia areas, *Epilobium hirsutum* L. blooms in July–August every year (Lee et al. 2018) and put forth up to 300–350 florets in an environment rich in water and 450–500 florets in an environment rich in organic matter (Lee et al. 2018). *Epilobium hirsutum* L. is designated as a rare plant in South Korea, an Asian area (NIBR 2014) because it inhabits wetlands with gravels and moist soil in streams and valleys and the wetlands as such are being destroyed due to human development (NIBR 2014).

Since plants store energy through photosynthesis and use the energy for growth and reproduction (Jansson et al. 2021), light quality can affect plant productivity (Yavari et al. 2021). The photosynthetic reactions according to light quality are regulated by various physiological variables including the leaf temperature, transpiration rate, photosynthetic quantum efficiency, and intercellular CO_2 partial pressure (Galle et al. 2009; Kim et al. 2013; Simon et al. 2018; Vilfan et al. 2019). However, the photosynthetic pathways of *Epilobium hirsutum* L. by light quality according to multivariate analysis have not been studied thus far, so that there have been difficulties in the studies of proliferation for the restoration of *Epilobium hirsutum* L. indoors. Therefore, in order to conserve the species of *Epilobium hirsutum* L., a rare plant in Korea, in a smart farm, this study conducted basic ecophysiology research according to light qualities using white light and controlling the ratio of blue light in a smart farm, which is a plant cultivation facility, with a view to collecting basic data for studies of *Epilobium hirsutum* L. proliferation in smart farms.

Materials and Methods

Cultivation of experimental plant

The *Epilobium hirsutum* L. individuals used in the experiment were those individuals that were similar in size selected in March 2020 from among those that were grown

in the greenhouse after receiving *Epilobium hirsutum* L. seeds from the Kicheongsan Botanical Garden in 2015. Three each of the selected seedlings were transplanted into each 36 cm wide, 26.5 cm long, and 21 cm high rubber water tank and one each of the rubber water tanks was placed under each light condition set inside the smart farm to cultivate the seedlings from March 2020 to August 2020. Thereafter, in order to prevent the leaves of individuals from overlapping with each other due to growth and development, from September 2020, 12 cm diameter and 15 cm high water tanks were filled with water, and one individual was transplanted into each of the water tanks and cultivated until December.

The size of the smart farm (Plant Factory System, Parus, Shanghai, China) used in this study is 582 cm wide, 334 cm long, and 260 cm high, and chambers that enables treatments with different light conditions were installed on the shelf inside the smart farm. The size of each of the chambers is 120 cm in width, 52 cm in length, and 41.5 cm in height, and LED panels are installed on the ceiling of each chamber.

In order to examine changes in the physiological and growth responses of *Epilobium hirsutum* L. according to light conditions, four chambers with different light conditions were installed in the smart farm. As for the selection of light conditions, red and blue lights, which are the most efficient for photosynthesis of plants, and white light, which has been frequently used in recent plant cultivation studies (Lee et al. 2019; Lee et al. 2020; Saleem et al. 2020) were selected. The LED monochromatic lights we used show the maximum number of photons at about 630–660 nm (red light), at 450 nm (blue light), and at 450 nm and 540 nm (white light), respectively.

Based on the foregoing, the light irradiation time ratios of blue light to red light in three chambers were set to 1:1 (T1), 1:1/2 (T2), and 1:1/5 (T3), respectively. The light irradiation time ratio of red light, blue light, and white light in the remaining one chamber was set to 1:1:1 (T4). In this case, the ratio of blue light to red light (B:R) was 0.64 in T1, 0.32 in T2, 0.13 in T3, and 0.36 in T4.

The temperature inside the plant factory was kept constant using a hot and cold air blower (SS-2000; Zero Engineering, Daejeon, Korea), and the humidity was kept constant using a humidifier. The amount of light in each chamber was adjusted using a computer so that a constant amount of light was maintained at the height of the water tank. The data on the physical environment inside the plant factory was transmitted to the computer at 30-minute intervals through the sensors installed inside each chamber.

The amount of light (mean ± standard deviation) in the smart farm during the experiment period was 107.98 ± 17.61 $\mu molm^{-1}s^{-1}$, the temperature (mean ± standard deviation) was 21.32 ± 3.96°C, the humidity (mean ± standard deviation) was 59.97 ± 12.94%, and the CO_2 concentration (mean ± standard deviation) was 379.86 ± 42.78 ppm. Because *Epilobium hirsutum* L. grows with its roots submerged in water in its habitat, the water level was always maintained so that the root zone was always submerged under water.

Measurement of physiological and growth responses

In the case of *Epilobium hirsutum* L., stolons used for vegetative propagation together with branches appear from the main stem (Fig. 1) and they were separated measuring physiological and growth responses because they have different purposes for development and their internal and external shapes of stems and leaves are different (Shamsi and Whitehead 1974). In order to identify changes in the physiological response of the leaves of *Epilobium hirsutum* L. according to light conditions, the temperature difference between the atmosphere and the leaves (ΔT; °C), the intercellular CO_2 partial pressure (Ci; vpm), the transpiration rate (E; $mmolm^{-2}s^{-1}$), and the net photosynthetic rate (Pn; $\mu molm^{-2}s^{-1}$) were measured with the leaves of the branch of *Epilobium hirsutum* L. in May 2020 using a photosynthesis measuring apparatus (LCi Ultra Compact Photosynthesis System; ADC BioScientific Ltd, Hoddesdon, UK), and the photosynthetic quantum efficiency for carbon fixation (Φ; $molCO_2mol^{-1}quanta$) was calculated by dividing Pn into incident light intensity on the leaf surface. When measuring the photosynthetic reaction, the temperature was 21.37 ± 0.99°C (mean ± standard deviation), the humidity was 56.88 ± 2.66% (mean ± standard deviation), and the CO_2 concentration was 349.08 ± 13.36 ppm. In addition, to identify changes in the growth response of *Epilobium hirsutum* L. according to light conditions, the length of the main stem of *Epilobium hirsutum* L. the number of leaves attached to the main stem, the numbers of aerial and stolons, and the lengths and the numbers of leaves per branch of the aerial and stolons were measured in December 2020.



Fig. 1 The shoot morphology (A) and the stolon stem (B) of *Epilobium hirsutum*. The white-colored arrows show the stolon leaves.

Statistical analysis

Factor analysis was carried out to identify major factors and variables for changes in the physiological response of *Epilobium hirsutum* L. according to the light environment (No and Jeong 2002). To that end, the observed values of the ratio of blue light to red light (B:R), the ratio of white light (W), and variables regarding physiological responses were standardized and factor scores were derived in order to minimize the effects of different units of variables on the analysis. In this case, the Varimax orthogonal transformation method was used as the orthogonal transformation method. In general, factor loading values derived from factor analysis will indicate that there are correlations if they are 0.3 or larger and will be considered as key variables if they are 0.5 or larger (Taherdoost et al. 2014). Factor analysis can analyze the correlations between light quality and physiological variables of *Epilobium hirsutum* L., but path analysis was performed to go further and identify the path of controlling the net photosynthetic rate of *Epilobium hirsutum* L. by light quality and the effects between individual variables.

Simple regression analysis was performed to identify the trend of the growth response of *Epilobium hirsutum* L. according to the changes in the ratio of blue light to red light. In addition, to examine the effect of white light treatment, the Mann–Whitney U-tests were performed between groups with the presence and absence of white light. Statistica software (StatSoft Inc. 2004) was used for factor analysis and simple regression analysis, and JASP software (JASP Team, 2021) was used for path analysis.

Results

Physiological response

According to the results of the factor analysis, the variables that showed correlations with factor 1 were W, ΔT, E, and Pn, and the variables that showed correlations with factor 2 were B:R, W, Ci, Pn, and Φ (Table 1). The key variables of factor 1 were W, ΔT, E, and Pn, and the key variables of factor 2 were B:R, Ci, and Φ (Table 1). The variables that commonly showed correlations with factor 1 and factor 2 were W and Pn (Table 1).

Table 1 The factor loadings on the 7 physiological variables of *Epilobium hirsutum* by factor analysis with varimax rotation method

Variables	Factor 1	Factor 2
B:R	–0.147	–0.690
W	0.630	0.324
ΔT	–0.761	–0.218
Ci	0.035	0.719
E	0.925	0.209
Pn	0.800	–0.302
Φ	–0.145	–0.759

B:R: amount of blue light per red light; W: amount of white light per red light; ΔT: difference between leaf and air temperatures; Ci: intercellular CO_2 pressure; E: transpiration rate; Pn: net photosynthetic rate; Φ: photosynthetic quantum efficiency on the incident light.

When the factor score distribution area for the four light conditions was divided into 4 groups according to individual light conditions, T1, which had the highest ratio of blue light to red light, was mainly located in the third quadrant, and T4 treated with white light was located in the first quadrant. Therefore, the physiological changes due to blue light and white light had opposite tendencies from each other (Fig. 1). T2, which had a lower blue light ratio and a relatively high red light ratio compared to T1, was mainly distributed in the second quadrant, and T3 was mainly distributed in the fourth quadrant (Fig. 1). However, the distribution area of T2 and T3 overlapped in part (Fig. 1).

According to the results of the path analysis, the increase in B:R increased ΔT (β = 0.25) and Φ (β = 0.54), and decreased E (β = –0.12) and Ci (β = –0.29) (Fig. 2). Conversely, the increase in W increased E (β = 0.29) and Ci (β = 0.7) and decreased ΔT (β = –0.31) and Φ (β = –0.17) (Fig. 2). ΔT affected by light quality decreased E (β = –0.71) and increased Ci (β = 0.23) (Fig. 2). In addition, Pn was increased by ΔT (β = 0.39), Φ (β = 0.27), and E (β = 0.54) and decreased by Ci (β = –0.83) (Fig. 2). Consequently, Pn was decreased by B:R (β = –0.33) and increased by W (β = 0.91) (Fig. 2).

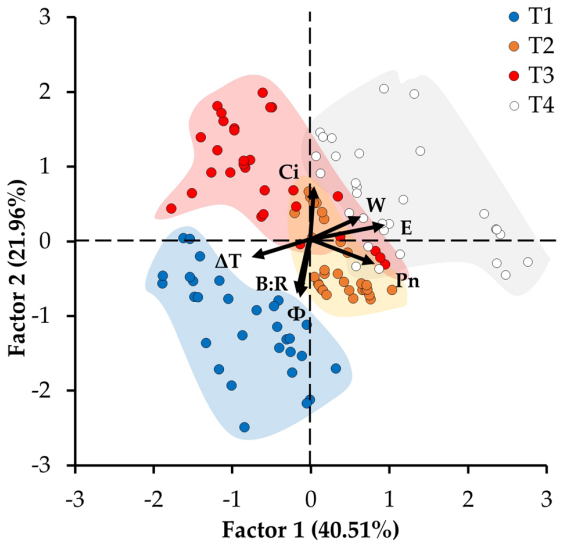


Fig. 2 Distribution of factor scores of T1, T2, T3, and T4 on the light quality and physiological variables, B:R, W, ΔT, Ci, E, Pn and Φ of *Epilobium hirsutum* by factor analysis with varimax rotation method. T1, T2, and T3 mean the irradiation time ratios of red + blue mixed light are 1:1, 1:1/2, and 1:1/5 respectively, and T4 mean the red + blue + white mixed light, the irradiation time is 1:1:1. The arrows mean factor loadings of each variable on the factor 1 and factor 2. B:R: amount of blue light per red light; W: amount of white light per red light; ΔT: difference between leaf and air temperatures; Ci: intercellular CO_2 pressure; E: transpiration rate; Pn: net photosynthetic rate; Φ: photosynthetic quantum efficiency on the incident light.

Growth response

According to the results of the simple regression analysis, the number of leaves of branches (β = 0.37) and the number of stolons (β = 0.35) of *Epilobium hirsutum* L. increased as the ratio of blue light to red light increased (Table 2). However, there were no differences in the number of branches and the length per branch, the length and number of leaves of the main stem, the length and number of leaves per branch of the stolons (Table 2).

According to the result of the Mann–Whitney U-tests, the numbers of the aerial and stolons of *Epilobium hirsutum* L. increased when treated with white light, compared to when only red and blue light were irradiated, and the number of leaves of the stolons increased (Table 3). However, there were no differences in the length and number of leaves of the main stem, the length and number of leaves per branch of the branches, and the length per branch of the stolons (Table 3).

Discussion

Physiological response

As the blue light ratio increased, the leaf temperature and photosynthetic quantum efficiency of *Epilobium hirsutum* L. increased (Figs. 2, 3). The leaf temperature can rise when the light absorption rate of the leaves is high (Muir 2019). Since photosynthetic quantum efficiency refers to the efficiency of photons participating in the carbon fixation reaction through photosynthesis with respect to the incident light on the leaves (Galle et al. 2009), it is related to the light absorption rate of the leaves. However, although light is absorbed by the leaves, not all of the absorbed photons are used for photosynthesis, and the light stress caused by the excessively absorbed light energy increases the leaf temperature due to the conversion of excessive light energy to thermal energy through the Xanthophyll cycle (Demmig-Adams and Adams 2006; Pollastri et al. 2014; Vilfan et al. 2019). Therefore, it is judged that the leaf temperature rose due to the light stress because although the photosynthetic quantum efficiency increases when the blue light increased, the excessive energy absorbed by the leaves also increases. However, since it is difficult to exactly determine the amount of light stress only by raising the leaf temperature, it is thought that a more accurate amount of light stress can be determined by measuring non-photochemical quenching (NPQ) through chlorophyll fluorescence experiments.

In contrast, when treated with white light, the leaf temperature and photosynthetic quantum efficiency of *Epilobium hirsutum* L. decreased (Figs. 2, 3). In a similar study, the photosynthetic quantum efficiency of blue light and red light was relatively higher compared to other wavelengths in roses (Paradiso et al. 2011), but the photosynthetic quantum efficiency of blue light decreased in lettuce (Liu and Van Iersel 2021). Therefore, photosynthetic quantum efficiency can be species-specifically affected by light quality. In addition, *Boehmeria nivea* L. showed relatively

Table 2 The result of simple regression analysis for growth responses of *Epilobium hirsutum* according to the changes in the ratio of blue light to red light

Groups	Variables	β	*p*-value
Plant	Branch number	–0.106	0.196
	Stolon number	0.346	≤ 0.001
	Main stem length	0.362	0.247
	Leaf number of main stem	0.503	0.095
Branch	Branch length	0.207	0.153
	Leaf number	0.365	0.010
Stolon	Stolon length	0.136	0.204
	Leaf number	0.169	0.114

β and p mean the regression coefficient and significant difference respectively.

Table 3 The average and standard deviation of growth responses of *Epilobium hirsutum*

Groups	Variables	BR	BRW
Plant	Branch number	3.83 ± 3.11†	10.69 ± 3.15†
	Stolon number	7.72 ± 3.27†	10.56 ± 2.97†
	Main stem length	64.11 ± 24.67	63.33 ± 16.04
	Leaf number of main stem	56.00 ± 40.53	53.67 ± 67.34
Branch	Branch length	12.39 ± 9.36	14.34 ± 9.28
	Leaf number	17.46 ± 11.51	16.24 ± 12.09
Stolon	Stolon length	24.49 ± 17.42	19.21 ± 13.43
	Leaf number	32.06 ± 21.37*	20.67 ± 17.90*

Values are presented as mean ± standard deviation.
BR: blue and red mixed light; BRW: blue, red, and white mixed light.
* and † mean significant differences, $p \le 0.01$ and $p \le 0.001$ respectively between BR and BRW analyzed by Mann–Whitney U-test.

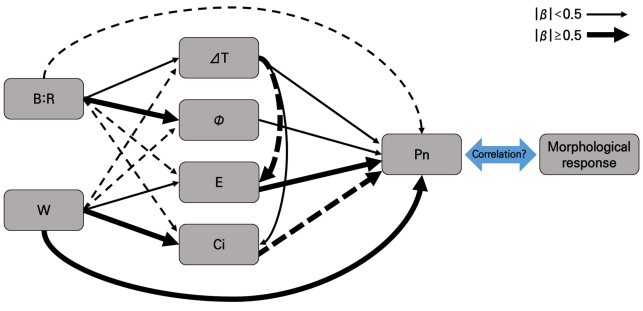


Fig. 3 Path analysis diagram for light quality and physiological variables of *Epilobium hirsutum*. The dotted and solid arrows mean the standardized regression coefficients are negative and positive values respectively. B:R: amount of blue light per red light; W: amount of white light per red light; ΔT: difference between leaf and air temperatures; Ci: intercellular CO_2 pressure; E: transpiration rate; Pn: net photosynthetic rate; Φ: photosynthetic quantum efficiency on the incident light. All regressions have significant differences, $p \le 0.05$. The weighted lines of the arrow mean standardized regression coefficient, $|\beta| \ge 0.5$. The photosynthetic rate also may correlate with morphological responses such as leaf or stem.

lower malondialdehyde (MDA) activity, which is used as a light stress index, in white light than in blue light, resulting in lower light stress (Rehman et al. 2020). Therefore, white light treatment is considered to have an effect to decrease light stress although its photosynthetic quantum efficiency is low.

The increase in leaf temperature and photosynthetic quantum efficiency increased the net photosynthetic rate (Fig. 3). However, the increase in the blue light ratio increased the leaf temperature and photosynthetic quantum efficiency while decreasing the net photosynthetic rate and white light treatment decreased leaf temperature and photosynthetic quantum efficiency while increasing the net photosynthetic rate. These results mean that the absorption of excessive light energy not used for photosynthesis increases when the ratio of blue light increases, and on the contrary, when white light is added, the absorption of excessive light energy relatively decreases.

Epilobium hirsutum L. decreased its transpiration rate when the blue light ratio increased (Figs. 2, 3). However, the transpiration rate increased when treated with white light (Figs. 2, 3). Since changes in leaf temperature can affect water retention in the body of leaves and the solubility of the CO_2 used for photosynthesis, when the leaf temperature changes, plants control the rate of transpiration through stomatal opening and closing (Henry et. al. 2019; Rangaswamy et al. 2021). Given the foregoing, the decrease in the transpiration rate following the increase in the blue light ratio is thought to be a phenomenon that occurred in the process of closing the stomata to keep the moisture inside the leaves constant against the heating due to excessive light stress. However, this decrease in the transpiration rate caused a loss of the photosynthesis rate (Fig. 3). This is because when stomatal pores are closed, the inflow of carbon dioxide used in photosynthesis decreases (Zhang et al. 2018). In addition, the increase in transpiration rate following white light treatment is thought to be intended to maintain the high net photosynthetic rate through the increase in transpiration rate with stomata opening because the plant became relatively safe from water loss due to evaporation of water as the leaf temperature was reduced (Fig. 3).

Meanwhile, blue light is known as a direct signal inducing stomatal opening (Inoue and Kinoshita 2017). *Vicia faba* induced stomatal aperture opening through the control of guard cells when red and blue light were treated together rather than when only red light was treated (Takemiya et al. 2006). However, since the activation of stomata opening by blue light is blocked by the abscisic acid (ABA) signaling mechanism produced under light stress (Driesen et al. 2020; Galvez-Valdivieso et al. 2009; Pospíšil 2016), in the case of *Epilobium hirsutum* L., it is thought that the signal for stomata opening is blocked even when the blue light ratio increased due to the high light stress. The decrease in the transpiration rate following the increase in the blue light ratio as such seems to consequently lead to a decrease in the net photosynthetic rate (Fig. 3).

In this study, an increase in the leaf temperature increased the intercellular CO_2 partial pressure. This result is thought to be related to photorespiration. Plants use photorespiration to directly use molecules such as ATP or NADPH thereby removing excess energy or increasing the amount of internal CO_2 in order to suppress the accumulation of harmful reactive oxygen species generated under light stress (Voss et al. 2013). In addition, when the leaf temperature rises, the solubility of CO_2 in water decreases, so that CO_2 cannot be used for photosynthesis and can be accumulated in the substomatal cavity. Therefore, it is judged that the net photosynthetic rate decreases as the intercellular CO_2 partial pressure increases (Fig. 3).

However, the increase in the blue light ratio increased the leaf temperature while simultaneously decreasing the intercellular CO_2 partial pressure and on the other hand, white light treatment decreased the leaf temperature while simultaneously increasing the intercellular CO_2 partial pressure (Fig. 3). Given that the increase in the blue light ratio consequently decreased the net photosynthetic rate of *Epilobium hirsutum* L. through decreasing transpiration rate, while the white light treatment increased the net photosynthetic rate through increasing transpiration rate, the changes in intercellular CO_2 partial pressure and net photosynthetic rate according to the light quality were more affected by the transpiration rate than by photorespiration.

Based on these results, when the blue light ratio increased, the photosynthetic quantum efficiency of *Epilobium hirsutum* L. increased, but light stress was induced, and the leaf temperature rose simultaneously. To keep the water content inside the leaves constant against the rise in leaf temperature as such, *Epilobium hirsutum* L. suppressed transpiration through stomatal closing. But, in this result, net photosynthetic rate was decreased. Under light stress, *Epilobium hirsutum* L. carried out photorespiration to suppress the accumulation of harmful reactive oxygen species, and in this process, the intercellular CO_2 partial pressure increased. When treated with white light, the photosynthetic quantum efficiency of *Epilobium hirsutum* L. decreased, but the light stress was reduced so that the leaf temperature decreased. Therefore, the transpiration rate could be increased for efficient photosynthesis.

Growth response

When the ratio of blue light to red light increased, the number of leaves of the branches of *Epilobium hirsutum* L. increased. However, there was no difference in the branch length. This means that the leaf density (number of leaves per unit length) of the branch increases as the blue light ratio increases. In a similar study, the number of leaves of Spinach, Kale, Basil, and Sweet Pepper increased as the ra-

tio of blue light to red light increased (Naznin et al. 2019). These plants also increased antioxidant activity as the blue light ratio increased (Naznin et al. 2019). Given that in the case of *Epilobium hirsutum* L., light stress is induced as the ratio of blue light increases, the increase in the number of leaves under blue light is thought to be related to light stress. Since this increase in the number of leaves as such can partially form shades where light is weak, it can increase the photosynthetic efficiency of the leaves located relatively lower in an environment where light stress is induced (Brouwer et al. 2012).

The number of branches of *Epilobium hirsutum* L. increased when treated with white light compared to when only red and blue light were irradiated. This is thought to be related to the lower light stress of *Epilobium hirsutum* L. in the light environment treated with white light. In a similar study, in the case of *B. nivea*, the higher the activity of MDA, superoxide dismutase (SOD), and peroxidase (POD) used as indicators of light stress, the higher the shoot fresh biomass and shoot dry biomass (Rehman et al. 2020).

In addition, there was no difference in the length and number of leaves of the branch of *Epilobium hirsutum* L. when treated with white light compared to when only red and blue lights were irradiated. This is because there was no need to increase the leaf density (number of leaves per unit length) since the light stress became lower when compared to the results for blue light. Therefore, when the light stress is high, *Epilobium hirsutum* L. tries to lower the light stress by forming partial shades through leaf production, but when the light stress is low, it does not produce unnecessary leaves but invests resources in the number of branches in an attempt to produce more photosynthetic products.

The increase in the blue light ratio increased the number of stolons of *Epilobium hirsutum* L. (Table 2). Therefore, blue light can induce colonization through the stolons of *Epilobium hirsutum* L. This has an ecologically significant implication, and colonization through stolons can be exposed to various light environments because it produces ramets, which are genetically identical individuals, and can spread horizontally (Guo et al. 2016). Therefore, species-specific phenotypic plasticity to cope with these diverse environments is important for the survival of ramets (Guo et al. 2016; Shen et al. 2020). In the case of *Amomum villosum* Lour., which produces ramets to propagate, when treated with various light doses, the stolon length was shortened and the number of ramets was small under intense amounts of light with high light stress (Guo et al. 2016). Therefore, *Epilobium hirsutum* L. seems to be able to rather expand its population by competitively producing ramets against other plants by increasing vegetative propagation in an environment where light stress is induced.

Treatment with white light increased the number of stolons similar to the increase in blue light ratio (Tables 2, 3). However, it produced a greater number of stolons than when only blue light was irradiated (Table 3). This is because more efficient production of photosynthetic products is possible by increasing the number of aerial steams when treated with white light than when treated with only blue light thanks to the lower light stress when treated with white light than when treated with only blue light. However, given that there is a tendency for the production of stolons to decrease when the ratio of blue decreases despite that the light stress decreases and the photosynthetic rate increases (Fig. 3 and Table 2), both light stress and blue light are judged to contribute to the production of the stolons of *Epilobium hirsutum* L.

This seems to be also related to the correlation between blue light and the net photosynthetic rate of *Epilobium hirsutum* L. Although the increase in the blue light ratio decreased the net photosynthetic rate overall but looking only at the results of T2 and T3, which are relatively rich in red light, the distribution area of factor scores for physiological responses was greatly affected by the change in the net photosynthetic rate, and the net photosynthetic rate of T3 with a lower blue light compared to T2 with a high blue light ratio was lower than that of T2 (Fig. 2). Therefore, when the blue light falls below a certain ratio compared to the red light, the stolon production may become difficult because the increase/decrease pattern of the net photosynthetic rate by the blue light is rather reversed.

In addition, the ramets of wild strawberry (*Fragaria vesca* L.) survived better even when exposed to a water-deprived environment when connected to their parent individuals (Roiloa and Retuerto 2005). This result shows that the survival is related to the mutual source-sink relationship through the stolon connection between the parent individuals and the ramets for the resource. In addition, individuals connected with each other through stolons may develop independent of each other, but functional specialization may occur (Stuefer 1998). Given the foregoing, *Epilobium hirsutum* L. is thought to seek an area where photosynthesis or resource utilization is stable by increasing the ramets through the number of stolons for survival in an environment where light stress occurs. Furthermore, stolon production seems to be carried out smoothly when the light stress is reduced because more photosynthetic products can be stably produced by increasing the number of branches.

The number of leaves of stolons decreased more when treated with white light than when treated with blue light alone. Unlike the leaves of branches, the leaves of stolons of *Epilobium hirsutum* L. are small in size and two of them are attached symmetrically on each node of the stolon. To date, no studies have been conducted on the leaves of the stolons of *Epilobium hirsutum* L. Therefore, their ecophysiological functions are not well known. However, given that the number of leaves of the stolons decreased in an

environment where light stress is reduced in contrast to an increase in the total number of leaves per individual due to an increase in the number of branches in the same environment, and that the leaves of a stolon can extend horizontally like an antenna along the stolon, it is thought that probably they play an ancillary role in the production of photosynthetic products used for vegetative propagation by being produced more and spread horizontally to try photosynthesis in an environment where photosynthesis is not performed well.

However, the length of stolons of *Epilobium hirsutum* L. was not affected by the increase in the blue light ratio and the white light treatment. Given that although the stolons can expand the population through vegetative propagation over relatively long distances when they are elongated, each ramets and the parents formed through the stolons can be functionally specialized, if the stolons are cut due to a certain event, the stolons may wither to death due to limiting factors in the environment where each individual is placed (Roiloa and Retuerto 2005). Therefore, when *Epilobium hirsutum* L. invests resources in the production of stolons for vegetative propagation, it seems that the *Epilobium hirsutum* L. is trying to increase the number of stolons rather than increasing the length of the stolons.

Unlike the results from the branches and stolons, the length and number of leaves of the main stem did not show any difference following the adjustment of the ratio of blue light to red light and white light treatment. When *Epilobium hirsutum* L. was grown in soil in Asia areas, the length of the aerial part was related to the contents of soil moisture and soil organic matter (Lee et al. 2017). Therefore, the length of the main stem is judged to be determined by the contents of soil moisture and soil organic matter rather than by light stress and the net photosynthetic rate. In addition, given that the number of leaves of the main stem did not appear to differ depending on the light conditions, it is judged that the main stem of *Epilobium hirsutum* L. mainly plays the role of absorbing water and organic matter from the roots and supplying them to other organs rather than the production of photosynthetic products through photosynthesis.

Based on these results, The leaves of the branch of *Epilobium hirsutum* L. were produced more to increase photosynthetic efficiency by forming partial shades in environments where light stress is high. In addition, more stolons were produced to escape from environments where light stress is high. Therefore, it seems that the population can be expanded more competitively in cases where light stress occurs in the habitat compared to other plants. In addition, it is judged that the stolons can perform the function of supporting photosynthesis in an environment away from the photoenvironment in which the parents are located by producing a large number of leaves of stolons in an environment unfavorable to photosynthesis.

However, *Epilobium hirsutum* L. produced more branches in order to produce more photosynthetic products in an environment where light stress is low resulting from treating white light. In addition, using the photosynthetic products produced as such, *Epilobium hirsutum* L. produced more stolons used for vegetative propagation. This means that the propagation of *Epilobium hirsutum* L. is mainly carried out through ramet production through stolons regardless of light stress. However, even if the light stress is reduced, if the net photosynthetic rate is lowered, ramets will not be produced well through the stolons. Given that unlike the aerial and stolons of *Epilobium hirsutum* L., the length and number of leaves of the main stem are not significantly affected even if the light conditions are changed, it is judged that the main stem mainly performs the function to transport water and nutrients to other organs such as branches and stolons rather than the production of photosynthetic products.

Conclusions

Epilobium hirsutum L. is a wetland plant limitedly distributed in areas where water supply is smooth, mainly in wet areas of the Northern Hemisphere. In Korea, which is an Asia area, *Epilobium hirsutum* L. was designated as a rare plant due to the destruction of its habitats. Although it was reintroduced as a restoration ecological method to keep the *Epilobium hirsutum* L. population stable in its native habitats, to that end, a measure to secure individuals with genetic diversity close to the parent population in the habitat in large quantities through proliferation including cultivation and vegetative propagation in artificial facilities is necessary. However, to that end, basic ecophysiological studies on *Epilobium hirsutum* L. should precede. Therefore, a basic ecological physiology study was conducted according to the light quality through the adjustment of the blue light ratio and the white light treatment in a smart farm.

As a result, when the blue light ratio increased, light stress was induced, resulting in a rise in leaf temperature, and the transpiration rate and net photosynthetic rate decreased due to stomatal closure. In this light condition, more branch and stolon leaves were produced due to light stress. On the contrary, when white light is treated, leaf temperature decreased by reducing light stress and increasing the transpiration rate and net photosynthetic rate. In addition, branch leaves were produced for efficient photosynthesis. Low light stress and high net photosynthetic rate increased the number of stolons used for vegetative propagation.

In this study, the effects of the ecophysiological response of *Epilobium hirsutum* L. on the growth and vegetative propagation were considered, but, in addition to the fore-

going, the morphological modification of external organs such as leaves and stems can affect physiological variables such as transpiration (Graham and Nobel 2005; Leigh et al. 2017). Therefore, in order to clearly understand the change in the net photosynthetic rate of *Epilobium hirsutum* L. according to the light quality, additional studies on the effect of biologic variations as such on the net photosynthetic rate are thought to be necessary (Fig. 3).

To put together the above results, in the case of *Epilobium hirsutum* L., when red and blue lights are supplied in mixture, the higher the ratio of blue light, the higher the light stress, but the production of stolons for vegetative propagation increases. However, when white light is mixed with red light and blue light to treat *Epilobium hirsutum* L, light stress is reduced, and the net photosynthetic rate is increased so that growth reaction through branch and leaf production and vegetative propagation through stolon production occur actively. Therefore, in order to induce vegetative propagation of *Epilobium hirsutum* L. through light quality in the smart farm, rather than increasing the ratio of blue light to red light, mixing white light with red light and blue light to treat *Epilobium hirsutum* L. is more efficient for vegetative propagation of *Epilobium hirsutum* L.

Abbreviations

ICT: Information communication technologies
IoT: the Internet of things
LED: Light-emitting diode
PPFD: Photosynthetic photon flux density
ABA: Abscisic acid
MDA: Malondialdehyde
SOD: Superoxide dismutase
POD: Peroxidase
CO_2: Carbon dioxide
ATP: Adenosine triphosphate
NADPH: Nicotinamide adenine dinucleotide phosphate

Acknowledgements

Not applicable.

Authors' contributions

YHY conceptualized the research and did experimental design. JHP and JML collected data. JHP analyzed data. JHP prepared first draft of the manuscript. JHP, EJK, JML, and YHY revised and finalized the manuscript.

Funding

This research was supported by the National University Development Project by the Ministry of Education in 2020 (Grant No: 2020-0727-01).

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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