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Variation of Saponarin Content in Barley Sprouts (*Hordeum vulgare* L.) by Natural Light Shielding: Implication of the Importance of Light Intensity

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ABSTRACT: Saponarin is a functional metabolite produced by barley sprouts, and the mass production of saponarin by this crop is attractive for dietary supplement manufacturing. Light is the most important environmental factor determining plant growth, survival, and the production of secondary metabolites including flavonoids. This study was conducted to investigate the importance of light intensity for saponarin production in barley sprouts using a hydroponic growth system. Light intensity was manipulated by using shielding treatments to 100, 80, 70, and 50% natural sunlight (NS), and crop cultivation was performed on a monthly cycle. We found that the growth rate and biomass of barley sprouts did not differ in response to the shield treatments, whereas the saponarin content did. The highest saponarin content (i.e., from 1329 to 1673 mg 100 g^{-1}) was observed in the 100% NS treatment, and it gradually decreased as light intensity also decreased. Statistical analysis revealed a significant polynomial relationship of saponarin content with cumulative PPFD (R^2 = 76%), implying that the absolute total amount of light exposure over the growth period has a large effect on saponarin productivity in a hydroponic facility. Taken together, our results showed that shielding conditions, which are often unintentionally created by the design of cultivation facilities, can adversely affect saponarin production in barley sprouts. In addition, it was confirmed through our findings that light conditions with at least 70% NS in the cultivation facility enable the production of an amount corresponding to the saponarin content of the sprouts (>1000 mg 100 g^{-1}) produced in the open field. Further studies are needed to investigate the underlying physiological and molecular mechanisms responsible for the relationship of saponarin content with light quantity and quality in barley sprouts.

1. INTRODUCTION

Light is a critical environmental factor governing physiological and biochemical processes that are integral to plant growth, development, and productivity. The quality and quantity of functional metabolites in crops are particularly dependent on light conditions.[1,2] Thus, it is important to provide sufficient light intensity to realize crop quality and productivity during cultivation. Many studies have reported that excessive or insufficient light conditions can inhibit photosynthesis, thereby impairing plant growth as well as the production of major biomolecules, including sugar, vitamin C, amino acids, and phytohormones.[3−5] In addition, inappropriate light environments can also lead to an increase in reactive oxygen species, such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($HO^•$), all of which interfere with the photosynthetic metabolism of plants.[6,7] Consequently, this can cause serious decreases in crop quality and yield.

Barley (*Hordeum vulgare* L.) is a major crop worldwide, ranking fourth in global food production. The barley cultivation area has been expanding over the world for a long time due to its high capacity to adapt to diverse environments.[8] Currently, growing barley sprouts is receiving great attention, especially in South Korea and other Asian countries, because it can be used as a raw material of health substances and dietary supplements.[9] So far, many studies have been revealed the pharmacological effects of barely sprouts on diabetes, hypertension, and antioxidants by beneficial compounds, including flavonoids, policosanols, lutonarin, saponarin, etc.[10−12] Furthermore, with the recent increase in demand for health foods, the efficacy of barley sprouts is becoming more popular.

In food crops, flavonoids are major phenolic compounds and significantly affect antioxidant properties, color, and aroma. They are therefore crucial determinants of crop quality and economic value.[13] However, the production of flavonoid compounds can vary greatly in response to many environmental factors, including light.[14−20] Saponarin is a flavone glucoside and constitutes the majority (ca. 70%) of the total polyphenol content of barley sprouts.[21−23] Saponarin is generally synthesized in the phenylalanine metabolism pathway, starting with naringenin synthesis catalyzed by chalcone synthetase (CHS) and chalcone isomerase (CHI). Naringenin is then converted to isovitexin, which is rapidly converted to saponarin by UDP-Glc:flavone-7-*O*-glycosyltransferase

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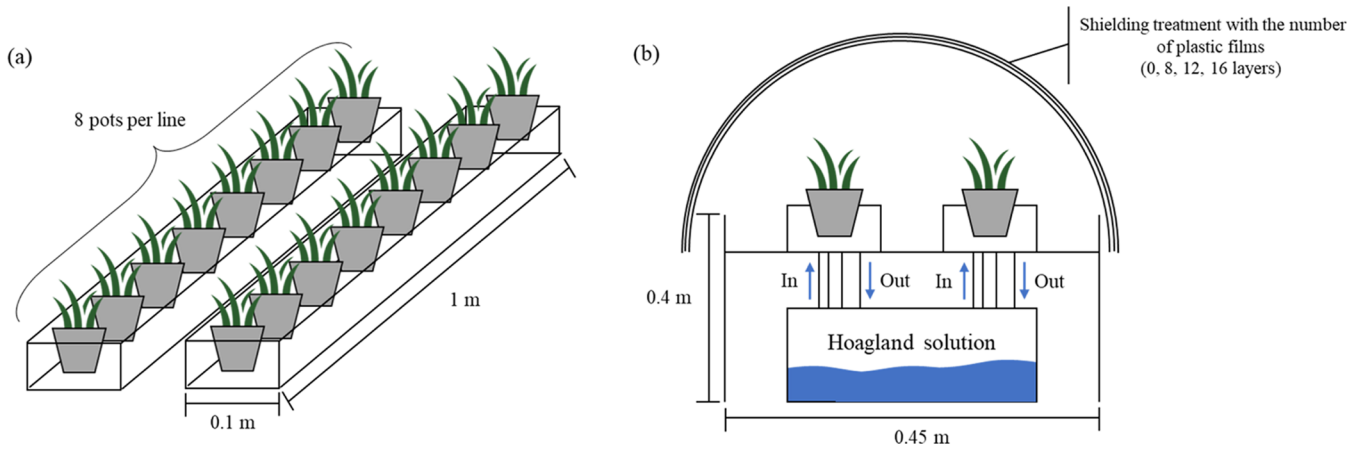

Figure 1. Hydroponic system with the Hoagland solution used in this study. The hydroponic cultivation system is composed of 16 pots in two lines (a) and the number of plastic films and corresponding light intensities for each setting is as follows (b): No layer—100%, 8 layers—80%, 12 layers—70%, and 16 layers—50%.

Table 1. Temporal Changes in Biomass and Growth Period of 15 cm Barley Sprouts Grown under Different Shielding Treatments[a]

	fresh weight (g plant^{-1})				growth period (day)			
treatment (% of natural light)	Jun	July	Aug	Sep	Jun	July	Aug	Sep
100%	0.157[a]	0.154[a]	0.154[a]	0.164[a]	9	10	6	10
80%	0.160[a]	0.156[a]	0.154[a]	0.169[a]	7	8	6	10
70%	0.160[a]	0.164[a]	0.156[a]	0.168[a]	7	8	6	10
50%	0.173[a]	0.158[a]	0.165[a]	0.166[a]	7	8	6	10

[a]Data presented are means of three replicates and the same letters indicate no significant difference in each column (Tukey's HSD, $p < 0.05$).

(OGT).[24] To date, many studies have demonstrated that there is a strong correlation between saponarin content in barley sprouts and light intensity and/or photoperiod, which strongly suggests that light conditions may be important for saponarin production.[12,25−28] Moreover, plant saponarin content can be elevated in response to various abiotic stresses, such as light, temperature, and plasma exposure, and it may also mitigate oxidative stress by acting as an antioxidant.[26−32] However, since most studies have been conducted in controlled environments, the influence on flavonoid biosynthesis and production by in situ environmental factors remains unclear.

Natural sunlight (NS) is an absolute determinant of crop growth and production, both in fields and in controlled cultivation, and the quality of sunlight reaching crops greatly impacts the generation of many plant compounds, including saponarin. Thus, attempts to mass produce saponarin-rich barley sprouts by identifying and implementing optimal light conditions will help establish a high-efficiency and high-income agricultural production system for this crop. In South Korea, most commercial barley sprout production is performed in outdoor fields, but this production environment may not be economically feasible. For example, in a preliminary study, we found that the production of high-saponarin barley sprouts in upland fields during the summer season (i.e., from June to August) was inefficient.[12] This was attributed to high levels of precipitation and cloudy weather, which contributed to low light intensity levels over the cultivation period. Given these facts, a cultivation facility that can consistently harvest saponarin-rich barley sprouts throughout the year may be an attractive alternative. This study was conducted to examine the primary impact of differences in natural light intensity on the saponarin content in barley sprouts grown using a hydroponic system.

2. MATERIALS AND METHODS

2.1. Plant Materials and Hydroponics. Seeds of the *H. vulgare* L. cultivar "Keunalbori 1" were obtained from the Rural Development Administration of the Republic of Korea. After soaking in distilled water for 1 day, seeds were germinated in the dark for 24 h. Next, germinated seeds were planted in 0.15 L pots containing perlite and then transferred to a hydroponic apparatus (Figure 1). The sowing rate was approximately 60 seeds (2.4 g) per pot, corresponding to a planting density of 8.4 t ha^{-1} in upland fields. The hydroponic system was installed outdoors at the farming facility of Gyeongsang National University, Republic of Korea (35°09′06.0″N 128°05′48.6″E). A modified Hoagland solution consisting of 2 M KNO_3, 1 M $Ca(NO_3)_2{\cdot}4H_2O$, 0.04 M Fe-EDTA, 2 M $MgSO_4{\cdot}7H_2O$, 1 M NH_4NO_3, 0.05 M H_3BO_3, 0.01 M $MnCl_2{\cdot}4H_2O$, 1 M $ZnSO_4{\cdot}7H_2O$, 0.2 mM $CuSO_4{\cdot}5H_2O$, 0.5 mM $Na_2MoO_4{\cdot}2H_2O$, and 1 M KH_2PO_4 (adjusted to pH 6.0)[33] was used as the growth medium. The properties of the hydroponic solution are listed in Table 1. Barley sprout cultivation was conducted monthly from June to September 2022.

2.2. Light Intensity Treatments. To adjust light intensity, a shielding (i.e., light-blocking) treatment was applied using different numbers of translucent polyethylene film sheets with a thickness of 0.1 mm as shown in Figure 1. During the entire experiment, the length of the day in each month was similar at 12 to 13 h. Shield treatments adjusted light received by plants to 100, 80, 70, and 50% of the full NS intensity consisting of 0, 8, 12, and 16 film layers, respectively. The plastic films also

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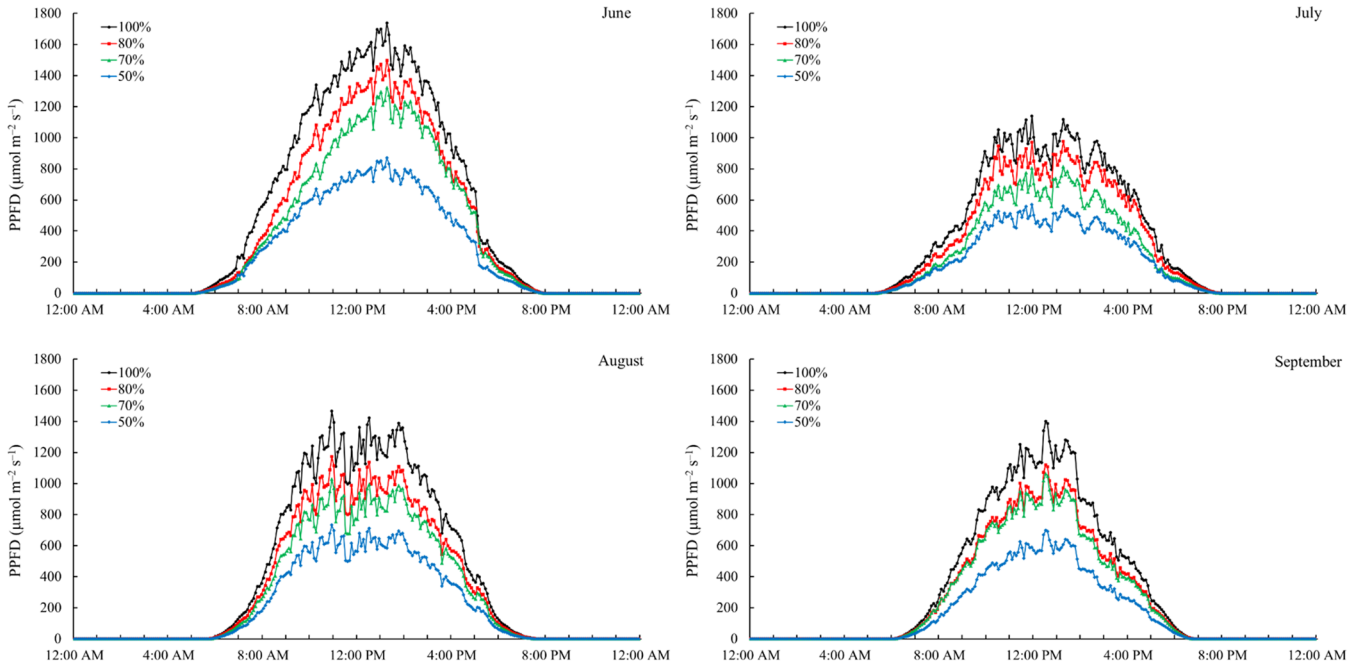

Figure 2. Temporal variation in photosynthetic photon flux density (PPFD) under different light intensity treatments: 100% (black), 80% (red), 70% (green), and 50% (blue) of natural light treatments.

modulated the wavelengths of transmitted light within the photosynthetic action spectrum, especially below 470 nm and above 650 nm (Figure S1, Supporting Information). Each light intensity treatment consisted of three technical replicates in the form of a hydroponic apparatus, and each replicate contained 16 pots in two lines. During the growth period, light intensity was measured every 10 min using a photosynthetically active radiation (PAR) sensor (SQ-521 PAR, METER group, Pullman) equipped with a ZL6 data logger. The light spectra were also determined by using a light monitor (International Light, RPS900-R, MA). All data collected were used to compute the cumulative photosynthetic photon flux density (PPFD) per duration (Cum. PPFD) and the average PPFD per day (Ave. PPFD) as the following equations. Since the values of PPFD (μmol m^{-2} s^{-1}) were collected at 10 min intervals, it was applied to the two equations after converting the total PPFD value for 10 min.

$$\text{Cum. PPFD (mol m}^{-2}\text{ duration}^{-1}\text{)} = \sum [\text{PPFD } (\mu\text{mol m}^{-2}\text{ s}^{-1}) \times 60\text{ (s)} \times 10\text{ (min)}]$$

$$\text{Ave. PPFD (mol m}^{-2}\text{ day}^{-1}\text{)} = \sum [\text{PPFD } (\mu\text{mol m}^{-2}\text{ s}^{-1}) \times 60\text{ (s)} \times 10\text{ (min)}] / \text{growth period (days)}$$

2.3. Plant Sampling. When the distance from the coleoptile to the true leaf of barley sprouts reached 15 cm in length, the upper part of the plant leaf sheath was sampled. Sprouts were harvested from 12 pots of each hydroponic apparatus, excluding the four pots at either end. The growth period and fresh weight (FW) of the plant samples were recorded at harvest. The FW was measured for plants in the 12 pots selected from one replicate of each shielding treatment. Samples were immediately freeze-dried at −50 °C for 5 days using a vacuum freeze-dryer (HyperCOOL HC3110, Hanil Scientific Inc., Gimpo, Korea). Freeze-dried samples were pulverized by a high-speed grinder (HR3757/00, PHILIPS, Amsterdam, Netherlands), passed through a 100-mesh sieve with a pore size of 149 μm, and stored at −20 °C until saponarin analysis was performed.

2.4. Quantification of Saponarin. Saponarin extraction from barley sprouts was performed using the method described by Seo et al.[23] These measurements were used to quantify how saponarin production varied with the cultivation time. To do so, 1 g of homogenized sample was added to 20 mL of 50% ethanol (v/v) and then incubated in a 35 °C experimental chamber for 24 h. The supernatant was then centrifuged at 7,800 rpm for 10 min before being filtered through a 0.2 μm polytetrafluoroethylene syringe filter (Whatman, Maidstone, U.K.). The saponarin content of the extract was determined via a Dionex Ultimate 3000 ultrahigh performance liquid chromatography (Thermo Scientific, Waltham, MA). A Zorbax Eclipse XDB-C18 column (i.e., 4.6 mm × 150 mm, 5 μm) was used with 35 °C set as the column temperature. Gradient mobile phases were prepared with 0.1% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B). The flow rate was 0.5 mL min^{-1} using the following gradient program: 3% B for 0−3 min; 3−15% B for 3−10 min; 15−30% B for 10−13 min; 30−50% B for 13−15 min; 50−90% B for 15−16 min; 90% B for 16−18 min; and 100% B for 18−20 min. The UV chromatogram wavelength was set to 325 nm with 10 μL of injection volume.

2.5. Statistical Analysis. Data reported in the present study indicate the mean (±standard deviation) of three parameters, saponarin content, FW, and PPFD. To compare parameter differences among different shielding treatments, we performed one-way ANOVA with Tukey's honestly significant difference posthoc tests at the 0.05 probability level (n = 3). Polynomial regression analysis was then used to evaluate the relationship between saponarin content and Ave. PPFD or Cum. PPFD. All statistical analyses were performed using R version 4.0.4 (R Foundation for Statistical Computing, Vienna,

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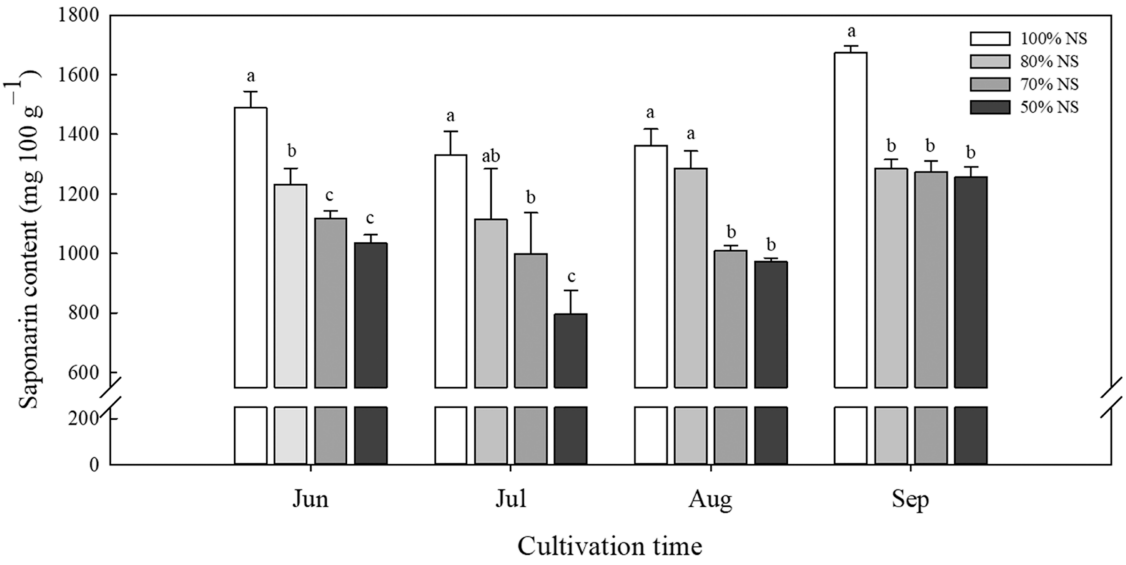

Figure 3. Saponarin content of barley sprouts grown under different light intensities, including 100, 80, 70, and 50% natural light treatments. Data indicate mean ± standard deviation of saponarin content in the sprouts produced with different cultivation times. The same letters in the same sowing season indicate no significant difference in saponarin content (Tukey's HSD, $p < 0.05$).

Table 2. Differences in the Cumulative PPFD and the Daily Average PPFD during Barley Sprout Cultivation among Shielding Treatments[a]

	cumulative PPFD (mol m^{-2} duration^{-1})				average PPFD (mol m^{-2} day^{-1})			
treatment (% of natural light)	Jun	July	Aug	Sep	Jun	July	Aug	Sep
100%	434.0^{a}	311.1^{a}	253.6^{a}	285.3^{a}	43.4^{a}	28.3^{a}	36.2^{a}	25.9^{a}
80%	261.8^{b}	213.1^{b}	202.9^{b}	228.2^{b}	32.7^{b}	23.7^{b}	29.0^{b}	20.7^{b}
70%	234.8^{c}	172.3^{c}	174.1^{c}	217.9^{b}	29.4^{c}	19.1^{c}	24.9^{c}	19.8^{b}
50%	171.8^{d}	91.3^{d}	136.7^{d}	223.3^{b}	21.5^{d}	10.1^{d}	19.5^{d}	20.3^{b}

[a]Data presented are means of three replicates and the same letters indicate no significant difference in the same column (Tukey's HSD, $p < 0.05$).

Austria). All graphs were generated using SigmaPlot version 12.0 (Systat Software Inc., San Jose, California).

3. RESULTS

3.1. Variation in Light Intensity. We found that the shielding treatments differed in terms of the PPFD values of NS (Figure 2). The highest PPFD during the experiment was observed in the 100% NS treatment, followed by 80, 70, and 50% NS treatments, respectively. Typically, the highest daily PPFD values were recorded between 11 am and 2 pm. In addition, we also found a temporal difference in mean PPFD values among different cultivation times, i.e., over the period from June to September. This effect is likely due to seasonal climatic characteristics, especially during the monsoon season between July and August.

3.2. Differences in Sprout Biomass and Growth Period. In June, the highest sprout FW was observed in the 50% NS treatment (0.173 g plant^{-1}), whereas the 100% NS treatment showed the lowest value (0.157 g plant^{-1}). However, we found no significant differences in sprout FW among the shielding treatments ($p > 0.05$; Table 1). Moreover, this trend was consistent across cultivation times. In addition, with respect to the duration of the growth period from zero to 15 cm, the 80, 70, and 50% NS shielded treatments showed periods that were several days shorter than those of the 100% NS treatment in June and July. In contrast, we observed no difference in the growth period for the remaining cultivation times (Table 1).

3.3. Saponarin Content. As shown in Figure 3, the saponarin content of the barley sprouts varied with the shielding treatment. In June, the highest saponarin content was found in the 100% NS treatment (i.e., ∼ 1500 mg 100 g^{-1}), followed by the 80, 70, and 50% NS treatments. Similarly, we also observed a decrease in saponarin with decreasing light intensity for the other cultivation times. In addition, sprout saponarin content differed among cultivation times; specifically, we observed the highest content in September, followed by June, August, and July, respectively. In all samples, the highest saponarin content (1673 mg 100 g^{-1}) was detected in sprouts grown under 100% NS in September, while sprouts grown at 50% NS in July produced the least saponarin (i.e., 796 mg 100 g^{-1}). Furthermore, the overall reduction rate of each shielding treatment relative to the 100% NS treatment (control) was as follows: vs 80% NS (a reduction of 17−31%), 70% NS (25−41%), and 50% NS (29−48%).

3.4. Relationship between Light Intensity and Saponarin Content. Table 2 shows the differences in cumulative PPFD among the shielding treatments. The highest Cum. PPFD in June was observed in the 100% NS treatment, followed by the 80, 70, and 50% NS treatments, respectively. This trend was also found for the other cultivation times. The Ave. PPFD values showed a tendency similar to that of the Cum. PPFD values, but we also observed slight differences related to differences in the growth period among cultivation times. Moreover, the polynomial regression test indicated that the sprout saponarin content was significantly associated with

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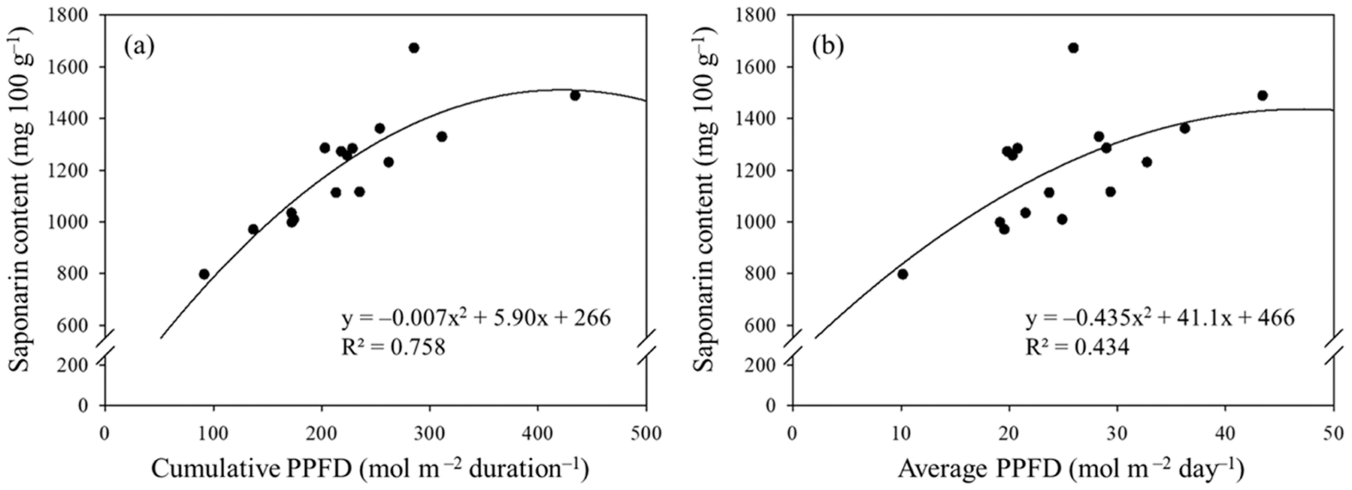

Figure 4. Polynomial relationship of saponarin content with cumulative PPFD (r^2 = 0.7576, p < 0.01) (a) and average PPFD (r^2 = 0.4339, p < 0.05) (b).

both Cum. PPFD (r^2 = 0.758, p < 0.01) and Ave. PPFD (r^2 = 0.434, p < 0.05) (Figure 4).

4. DISCUSSION

Due to the growing commercial interest in the production of supplements derived from natural substrates, including from plant secondary metabolites, there is also considerable interest in developing production techniques for enhancing the nutritional or medicinal values of target crops. Because barley sprouts contain high levels of functional compounds, including flavonoids, lutonarin, and saponarin,[11,32] they have become a major crop for the production of dietary supplements, especially in South Korea. Among the metabolites of interest present in barley sprouts, saponarin is the most important, possibly due to its use for treating various diseases, including carcinogenesis, hypocholesterolemia, and inflammation.[12,23,26] However, the biosynthesis of saponarin in sprouts varies greatly among cropping systems and environmental conditions, including temperature, water, and light intensity.[34,35] In an earlier study, we confirmed that seasonal variation in both daily temperature difference and photoperiod strongly affects saponarin production in barley sprouts grown in in situ open fields.[12] In contrast, to date, no studies have examined indoor facility cultivation environments that may be better able to regulate the mass production of sprouts with high saponarin content.

Greenhouse cultivation facilities are agricultural structures equipped with irrigation, drainage, and temperature control systems that enable efficient and intensive agricultural production. However, while these facilities are usually covered in transparent materials to let in NS for plant growth, spatially shielded environments can often be created, which can adversely affect crop quality and yield.[36] Here, we found that the highest saponarin content of barley sprouts was found in the 100% NS treatment at all cultivation times, and we also clearly observed a decrease in saponarin content as the light intensity decreased (Figure 3). This finding suggests that saponarin production is strongly dependent on light intensity. In particular, we found that the 50% shielding treatment reduced saponarin content by 25−40% compared to the full NS treatment over the entire growth period, thereby demonstrating that saponarin production can be significantly impaired by a reduction in NS.

According to previous studies, flavonoid biosynthesis is related to light-dependent photosynthetic reactions.[37,38] Ye et al.[39] also reported a decrease in flavonoid biosynthesis (i.e., flavonol glycoside) in young leaves of the tea plant (*Camellia sinensis* L.) in response to shade treatments. In that system, the shade created by black nets altered photosynthesis, thereby changing the composition of the sugar moiety by decreasing monoglucoside and diglucoside content and increasing triglucoside content.[39] Since saponarin contains two molecules related to glucose, the specific sugar moieties generated via light-dependent photosynthesis may determine the rate of saponarin biosynthesis in barley sprouts. Moreover, it has been confirmed through a molecular study that light conditions affect the gene expression patterns related to saponarin biosynthesis; the transcriptional activities of *HvCHS1*, *HvCHI*, and *HvOGT1* genes in barley sprouts decreased as the photoperiod decreased.[26] As such, in this study, it seems that the shielding effect inhibited the biosynthetic mechanism of saponarin, resulting in a decrease in the saponarin yield from barley sprouts.

Despite the obvious negative effects of shielding, the saponarin content of barley sprouts grown in most treatments exceeded 1000 mg 100 g^{-1}, which is higher than that typically found in sprout products sold in the Korean market.[40] However, here, we observed a single exception: the saponarin content of the 50% NS treatment in July, which showed a saponarin level of 797 mg of 100 g^{-1} (Figure 3). This may be attributed to the insufficiency of the light intensity (i.e., 200 μmol m^{-2} s^{-1}) experienced by these barley sprouts; this should be compared to the previously determined saturation point for barley plant photosynthesis of approximately 400 μmol m^{-2} s^{-1} PPFD.[41] Similarly, several studies have reported that barley sprouts grown at low light intensity (i.e., 380 μmol m^{-2} s^{-1}) using a chamber system contained low saponarin content (i.e., from 20 to 80 mg 100 g^{-1}).[25,42,43] However, under higher light intensities (i.e., 570−1,700 μmol m^{-2} s^{-1}) recorded in outdoor fields, saponarin content of barley sprouts ranged from 1000 to 1900 mg 100 g^{-1}.[12] Moreover, the authors of previous study observed a positive correlation between saponarin yield and light duration (r = 0.446, p = 0.006) but not light intensity (r = 0.095, p = 0.584).[12] Conversely, in this study, saponarin content in barley sprouts showed a significant polynomial relationship with Cum. PPFD (R^2 = 75.8%), confirming the

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strong link between light intensity and sprout saponarin production.[44] It is thought that the contradictory results of these two experiments are attributed to the difference in the applied cultivation seasons, which may differ significantly for various climatic factors, such as photoperiod, temperature, etc.

Overall, we consider natural light intensity to be a key determinant of saponarin production in barley sprouts based on the results of our mesocosm experiment. In addition, we expect that our findings may be important for further maximization of the quality and quantity of saponarin production in barley sprouts by increasing the efficiency of customized light supply according to the variation among climate conditions. Furthermore, studies of the effect of light quality on saponarin production are also needed to establish a better growing system for the mass production of barley sprouts. Accordingly, numerous studies on the relationship between light quality and flavonoid production (including saponarin) have been recently conducted.[25,27,34,39,45,46] In particular, the positive effect of blue LED light illumination on saponarin production in barley sprouts was reported by Chung et al.[25] and Muthusamy et al.[45] This was due to the fact that blue light irradiation leads to upregulating the *HvOGT1* gene expression, which play a crucial role in enriching saponarin flavone.[9] In the present study, different light spectra according to the shielding degree were observed, especially in the blue region (400−470 nm) (Figure S1, Supporting Information), and thus, it seems that there was a difference in saponarin content among the treatments. In addition, differences in the photosynthetic activity by the light spectrum change might influence saponarin production in the sprouts.[9,47] Despite these insights, further studies are needed to investigate the underlying mechanism responsible for the close relationship between the light environment and the saponarin content of barley sprouts to optimize growing conditions for the production of high-value barley sprouts.

5. CONCLUSIONS

The present study is an early attempt to understand the importance of natural light intensities on the growth and saponarin production of barley sprouts grown in the hydroponic cultivation facility. Clearly, we did confirm a decreasing trend in saponarin content of barley sprouts as the natural light intensities decreased. However, some degree of moderate shielding (i.e., levels greater than 70% of natural light) appeared to be sufficient in terms of saponarin production, compared to the field-grown barley sprouts (>1000 mg 100 g^{-1}). In addition to the light intensity, the quality of natural light affected by shielding may be another crucial factor for saponarin production in the sprouts. Our findings can highlight the importance of natural light transmittance for the sustainable mass production of saponarin-rich barley sprouts in indoor facilities. Further studies of physiological and molecular mechanisms related to our results are needed to increase the efficiency of barley sprout production in the future.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c03458.

Variations of the absolute irradiance according to the shielding treatments (Figure S1) (PDF)

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Author Contributions

‖Y.-E.Y. and J.Y.C. contributed equally to this manuscript. Y.-E.Y., W.D.S., and Y.B.L. designed the project. Y.-E.Y., J.Y.C., V.K., and K.-A.L collected the data. Y.-E.Y., Y.-N.K., V.K., and K.-A.L visualized all data. Y.-E.Y. and J.Y.C. wrote the first draft. Y.-N.K, K.-A.L., and Y.B.L. reviewed and edited the manuscript. All authors have read and approved the final draft of the manuscript.

Notes

The authors declare no competing financial interest.

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