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Effect of continuous lighting on the growth and leaf chemical components of *Artemisia princeps* grown hydroponically in a plant factory condition

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Abstract: Young leaves of *Artemisia princeps* Pamp. (Japanese mugwort), already used as a foodstuff in Japan, can be positioned as a functional health food because of remarkably higher contents of chlorogenic acid and total polyphenol compared to common vegetables. To procure young leaves in demand on a year-round basis by hydroponic production in fully artificial light-type plant factories, we investigated whether 24-h photoperiod, known to enhance some beneficial constituents, could improve the growth and chemical constituents of Japanese mugwort plants grown hydroponically in a plant factory condition. As we previously demonstrated that lowering the nutrient solution concentration increased chlorogenic acid and total polyphenol contents of the leaves without reducing the growth, plants were cultivated with a lower concentration of nutrient solution. The results indicated that it is possible to grow Japanese mugwort hydroponically under 24-h photoperiod in a plant factory condition with a nutrient solution concentration as low as 25% of the standard. In addition, under 24-h photoperiod, plant growth was greatly accelerated and chlorogenic acid as well as total polyphenol were increased, suggesting that 24-h photoperiod is highly beneficial for Japanese mugwort production in a fully artificial light-type plant factory.

1. Introduction

Mount Ibuki, located on the border of Shiga and Gifu prefectures in Japan, has been famous for its medicinal plants since ancient times, and it was written in 'Engishiki' (compiled in 927 A.D.) that Omi (Shiga Prefecture) and Mino (Gifu Prefecture) ranked first and second, respectively, in the number of herbal medicinal items as paying tribute to the imperial court from all over Japan (Oda, 1985). In particular, in the early Edo era (around 1700 A.D.), the area around Mt. Ibuki was a major producer of domestic mugwort, such as *Artemisia princeps* or *Artemisia montana*, and the resulting moxa, called for 'Ibuki-Moxa' was publicized nationwide (Oda, 1998, 1999). The authors focus on the use of such

domestic mugwort.

Gaiyoh (Artemisiae folium) used in Wakan-yaku (traditional herbal drugs) is defined as the dried leaves and branch tips of *A. princeps* or *A. montana*, and it is used as a raw material for moxa and is included in various Chinese herbal preparations as an astringent hemostatic and analgesic (Nunome, 2018; Ministry of Health, Labour and Welfare, 2021). *Artemisia princeps* (Japanese mugwort) is also used as a foodstuff, with its young leaves, picked in early spring, being mixing with rice cakes or dumplings as 'Mochigusa', or used in soaking and tempura (Odachi and Hiyama, 2013; Ando *et al*., 2022). In particular, according to the Functional Components Database (National Agriculture and Food Research Organization, 2020), Japanese mugwort has remarkably higher chlorogenic acid and total polyphenol contents compared with common vegetables, indicating that it can be positioned as a functional health food.

Japanese mugwort is generally procured by harvesting wild plants or through cultivation in open fields (Ando *et al.*, 2022). It is preferable to harvest the young, tender leaves in early spring for use as a food ingredient or functional health food. However, under natural conditions, the number of mature leaves increases with plant growth, and after flowering in autumn, the plant eventually withers and stops growing until the following spring (Ito, 2015), making it difficult to procure young in-demand leaves on a year-round basis, even after harvesting both wild and cultivated plants. To address this problem, we focused on the use of a fully artificial light-type plant factory system. With the multi-shelf cultivation system used in plant factories (Kozai, 2013), it is possible to produce a large number of young plants at a low plant height on a year-round basis, allowing to provide the young leaves desired throughout the year (Kim *et al.*, 2021). In addition, plant factory production has the advantage of being pesticide-free.

However, to date, there is limited knowledge on the hydroponic cultivation of Japanese mugwort; therefore, it is necessary to establish an effective management system for its hydroponic cultivation in fully artificial light-type plant factories. In our previous report (Hata and Kawamura, 2021), we investigated the effects of nutrient solution concentration on the growth and leaf chemical components of Japanese mugwort cultivated hydroponically using 'Ibuki-yomogi' (a line of *A. princeps* indigenous to Shiga Prefecture), to establish an effective hydroponic cultivation method. The results showed that lowering the nutrient solution concentration to 25% of the standard increases the ascorbic acid, chlorogenic acid, and total polyphenol contents of the leaves without reducing plant growth.

Hata *et al*. (2012 a) studied the differences in the growth rate and leaf sesamin content of sesame (*Sesamum indicum*) grown under various photoperiods and found not only a maximum leaf yield, but also a distinctively high sesamin content, under a 24-h photoperiod. Furthermore, Higashiuchi *et al*. (2016) also reported that the active ingredient (asperuloside) level in white flower snake-tongue grass (*Hedyotis diffusa*), a medicinal plant, increases noticeably under a 24-h photoperiod compared with under 14- and 19-h photoperiods. Thus, enhanced leaf yields and accumulations of beneficial components may be achieved using a 24-h photoperiod in the cultivation of Japanese mugwort in a plant factory; however, supporting research is required.

Consequently, in the present study, we used 'Ibuki-yomogi' in our experiments and investigated whether a 24-h photoperiod increased the growth and chemical component contents of Japanese mugwort plants grown hydroponically in a low nutrient solution concentration under plant factory conditions.

2. Materials and Methods

Plant materials and seedling cultivation methods

The strain maintained at the Ibuki Yakuso-no Sato Cultural Center (Maibara-city, Shiga Prefecture, Japan) was used as the experimental material. Inflorescences collected in the fall of 2017 were air-dried and stored in a desiccator for use in the cultivation experiments.

The seedlings were grown in the growth chamber. The photosynthetic photon flux density from the Hf-fluorescent lamp (FHF32EX-N-H, Panasonic Co., Japan) on the surface of a seedling box was 260 µmol m^{-2} s^{-1}. The photoperiod and temperature were set at 12 h and 23°C, respectively.

On the basis of our previous report (Hata and Kawamura, 2021), seeds were spread by rubbing the flower heads with fingers, and then they were placed on root prevention sheets (20701FLD, Unitika Ltd., Japan) laid on Kim Towels (Nippon Paper Group Crecia Co., Ltd., Japan) moistened with tap water. At 1 week after sowing, young seedlings of approxi-

mately 3 mm were transplanted into polyurethane cubes (2.35 × 2.35 × 3 cm, Tomiyamass Co., Japan). Afterwards, the seedlings were grown for 3 weeks by subirrigation with 1/2-strength Enshi formula nutrient solution. This solution consisted of 2 mM of $Ca(NO_3)_2 \cdot 4H_2O$, 4 mM of KNO_3, 0.67 mM of $NH_4H_2PO_4$, 1 mM of $MgSO_4 \cdot 7H_2O$, 1.5 mg L^{-1} Fe, 0.25 mg L^{-1} Mn, 0.25 mg L^{-1} B, 0.025 mg L^{-1} Zn, 0.01 mg L^{-1} Cu, and 0.005 mg L^{-1} Mo.

Hydroponic methods

Hydroponic cultivation was conducted in a walk-in type plant growth room (internal dimensions: 4.1 m long, 4.1 m wide, and 2.1 m high) at the Experimental Agricultural Facility of The University of Shiga Prefecture. During the cultivation period, the temperature and CO_2 concentrations were set at 23°C and 400 ppm, respectively, while the relative humidity was not set at a constant level. The photosynthetic effective photon flux density on the surface of the growing container at a distance of 42 cm vertically from the Hf-fluorescent lamps (FHF32EX-N-H, Panasonic Co., Japan) was 130 µmol m^{-2} s^{-1}.

The seedlings were planted at 4 weeks after sowing and grown hydroponically for 4 weeks under a 12-h or 24-h photoperiod. A 2.5-cm thick Styrofoam board with two 2.5-cm diameter holes (11 cm between plants) was floated as a planting board on 6.0 L of nutrient solution in each container (NF Box #11 Blue, inner dimensions: 15.3 × 27.8 × 16.5 cm, capacity: 7.0 L, JEJ Astage Co., Ltd., Japan), and two seedlings were planted per board. The nutrient solution used was 1/4-strength Enshi formula, which was continuously aerated at 0.4 L min^{-1} with an air pump. The initial pH of the nutrient solution was adjusted to 6.0 with H_2SO_4 before use, but it was not adjusted during the cultivation period. The nutrient solution was renewed after 2 and 3 weeks of hydroponic cultivation. The pH of the nutrient solution was measured with a digital pH meter (pH-208, Sato Shoji Co., Ltd., Japan) before nutrient solution replacement and at harvest (4 weeks after the start of hydroponic cultivation).

Growth evaluation and preparation of dry matter samples

In total, 20 plants were grown in 10 growing containers under each photoperiod. The plants were harvested at 4 weeks after the start of hydroponic cultivation, and the fresh weights of leaves, stems, and roots were measured, as were the main stem lengths and numbers of branches, for all the plants. The stems and roots were dried in an oven at 60°C, after which the constant dry weights were recorded and used for calculating dry matter content. Approximately 10-15 g of leaves randomly taken from the whole leaves was similarly dried at 60°C to form a dried sample for the inorganic component analysis as well as the dry matter content calculation. The rest of the leaves were freeze-dried for other component analyses and stored in a -80°C freezer.

Chemical composition analysis

The 60°C-dried and freeze-dried samples were thoroughly ground independently with a mortar and pestle. In each photoperiodic treatment, 10 samples were analyzed for each component, with one sample being a mixture of equal amounts of the two individuals growing in one container. Each analysis described below was conducted similarly in accordance with our previously reported methods (Hata and Kawamura, 2021).

Determination of inorganic components

For each sample, 100 mg of the powdered sample was decomposed using the wet method in a nitric acid and hydrogen peroxide mixture in a 100-mL beaker. After decomposition, the solution in the beaker was volumetrically diluted with 1 M nitric acid and passed through a 0.45-µm syringe filter (Surplux PTFE-H (hydrophilic) 25 mm, LMS Co., Ltd., Japan). The P, K, Ca, Mg, Na, Fe, Mn, and Zn concentrations were measured using (SII SPS3100, Hitachi High-Tech Science Co., Ltd., Japan). Multi-element standard IV and single-element standard (P) for ICP (Merck Millipore Ltd., Germany) were used as calibration standards, and the content of each inorganic component in the leaves was calculated from the intensity value of each sample.

Determination of ascorbic acid

For each sample, 50 mg of the powder, weighed in a 2-mL microcentrifuge tube, was extracted using ultrasonic waves for 30 min in distilled water. After extraction, the ascorbic acid content in centrifuged supernatant liquid was measured using a reflectometer (RQ Flex 10, Merck Millipore Ltd., Germany) to calculate the corresponding content in the leaves.

Determination of chlorogenic acid

For each sample, 50 mg of the powder, weighed in a 2-mL microcentrifuge tube, was extracted at

40°C for 30 min with shaking at 2,000 rpm in 80% (v/v) ethanol solution. After the extraction, the supernatant was collected by centrifugation at 12,500 rpm for 5 min, and the extract was collected again from the extraction residue. The collected extract mixture was passed through a 0.45-µm syringe filter (GL Chromato-Disk 4N, GL Sciences Inc., Japan) before being used for the chlorogenic acid concentration analysis with the UPLC-FLD method. In brief, samples were analyzed with the ACQUITY UPLC system (Waters Co., USA) using a Waters ACQUITY UPLC HSS T3 Column (100 mm × 2.1 mm, 1.8 µm). Detection was performed using a Waters 470 Scanning Fluorescence Detector set at an excitation wavelength of 371 nm and an emission wavelength of 443 nm. The mobile phases were 0.2% (v/v) formic acid (solvent A) and 100% acetonitrile (solvent B). The gradient elution program, with a mixture of solvents A and B, was as follows: 90-80% A for 0-1 min (curve no. 7), 80-55% A for 1-5 min (curve no. 7), 55-35% A for 5-6 min (curve no. 9), and 35-90% A for 6-7 min (curve no. 9). The flow rate was 0.3 mL min^{-1}. The column oven was set at 40°C, and 3 µL of each sample was loaded. The amount of chlorogenic acid in a sample was quantified from the peak area of the authentic standard compound (chlorogenic acid hemihydrate dissolved in 80% ethanol) to calculate the content in the leaves. Solvents of HPLC grade, and all other chemicals, were purchased from Nacalai Tesque, Inc., Japan.

Determination of total polyphenol

A 10-fold dilution of the extract solution for the chlorogenic acid analysis with 80% (v/v) ethanol was prepared and analyzed in accordance with the Folin–Ciocalteu method. First, 0.3 mL of the sample solution and 0.3 mL of distilled water were mixed in a 2-mL microcentrifuge tube, and then, 0.6 mL of a solution of phenol reagent (Nacalai Tesque, Inc., Japan) diluted two-fold with distilled water was added and left for 3 min after mixing. Next, 0.6 mL of 10% (w/v) sodium carbonate solution was added, mixed, and allowed to react for 60 min. Within 30 min of the reaction finishing, the absorbance at a wavelength of 750 nm was measured using a spectrophotometer. Chlorogenic acid hemihydrate dissolved in 80% ethanol was used as the calibration standard, and the total polyphenol content in the leaves was calculated as chlorogenic acid equivalents from the absorbance values (750 nm) of each sample.

Data analyses

For growth data, average values for each growing container were compared, whereas the data for the nutrient solution pH levels and chemical components were compared among the obtained values per growing container. Significant differences between two photoperiods were analyzed using Student's t-test (n = 10).

3. Results

Change in nutrient solution pH

After 2 weeks of hydroponic cultivation, the pH of the nutrient solution rose to 6.5 under 12-h photoperiod, while it rose to 7.7 in the 24-h photoperiod (Fig. 1). Even after returning to the initial pH of 6.0 by replacing the nutrient solution, the pH rose to only 6.5 under 12-h photoperiod but to 7.7 under 24-h photoperiod in the following week. Even when the nutrient solution was renewed again after 3 weeks of hydroponic cultivation, the pH rose to 6.6 under 12-h photoperiod but to 7.7 under 24-h photoperiod, at the end of cultivation one week later.

Plant growth

Flower buds did not form under either the 12- or 24-h photoperiod until the end of cultivation at 8 weeks after sowing. From 2 weeks after the start of

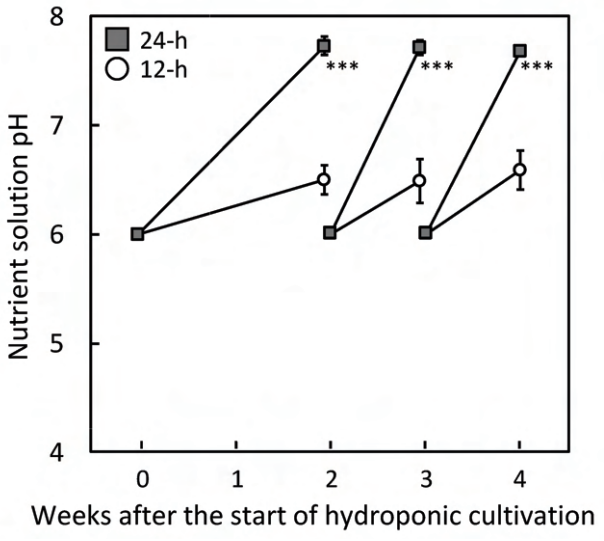

Fig. 1 - Changes in nutrient solution pH during the hydroponic cultivation of Japanese mugwort plants grown under 12-h (open circles) and 24-h (closed squares) photoperiods. Values represent means ± SEs (n = 10). Statistical significances between the two photoperiods were determined using Student's t-test. ***, $p < 0.001$.

hydroponic cultivation, plant growth was more vigorous under the 24-h photoperiod than under the 12-h photoperiod (Fig. 2). Generally, darker leaf colors and greater anthocyanin accumulations in the main stems were observed under the 24-h photoperiod (Fig. 3), and 2 of 20 plants showed lower leaf senescence (Fig. 4). There was no visual difference in the number of trichomes on leaves and stems between the two photoperiodic treatments (Fig. 3, 5).

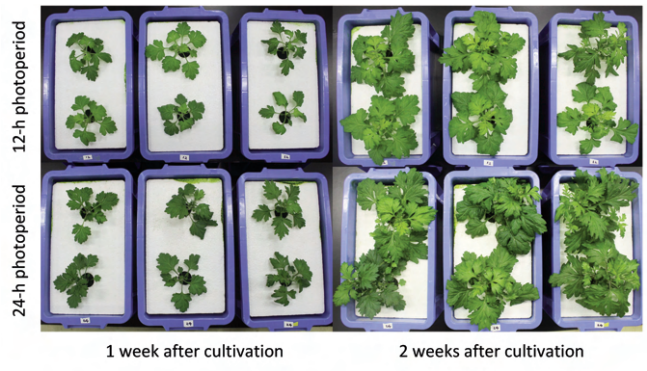

Fig. 2 - Differences in early developmental stages of Japanese mugwort plants grown under 12-h and 24-h photoperiods.

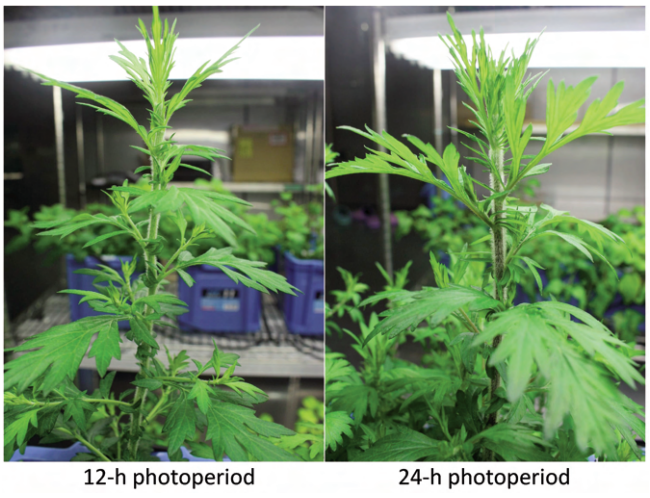

Fig. 3 - Differences in main stem colors of Japanese mugwort plants grown under 12-h and 24-h photoperiods for 4 weeks.

The fresh weights of leaves, stems, and roots at harvest under the 24-h photoperiod were 22.8, 11.1, and 14.6 g, respectively, which were almost twice as high as those under the 12-h photoperiod (Table 1). The dry weights of leaves, stems, and roots showed the same trends as fresh weights, and the dry matter ratio of leaves to stems was also significantly greater under the 24-h photoperiod. All the traits related to stem elongation, such as number of branches, main

Fig. 4 - Appearance of lower-leaf browning in the Japanese mugwort plant grown under a 24-h photoperiod for 4 weeks.

Fig. 5 - Appearances of trichomes on the abaxial leaf surfaces of Japanese mugwort plants grown under 12-h and 24-h photoperiods for 4 weeks.

stem length, number of main stem nodes, and average internode length, were significantly higher under the 24-h photoperiod compared with under the 12-h

Table 1 - Effects of photoperiod on the biomass production of Japanese mugwort plants

Photoperiod (h)	Leaves			Main stem + branches			Root		
	FW (g)	DW (g)	DMR (%)	FW (g)	DW (g)	DMR (%)	FW (g)	DW (g)	DMR (%)
12	13.132	1.4	11	3.7	0.4	10	8.0	0.6	8
24	22.81	3.0	13	11	1.4	12	15	1.0	7
Significance [z]	***	***	***	***	***	***	***	***	NS

FW= Fresh weight; DW= Dry weight; DMR= Dry matter ratio.
[z] Statistical significances between the means of two photoperiods were determined using Student's t-test (n = 10). NS= not significant; ***, p<0.001.

photoperiod (Table 2).

Inorganic component contents

On a dry weight basis, the K and Zn contents were significantly lower at the 1% significance level under the 24-h photoperiod compared with under the 12-h photoperiod (Table 3).

Furthermore, the Fe and Mn contents were also significantly lower under the 24-h photoperiod at the 0.1% significance level. The P, Ca, Mg, and Na contents were not significantly different between the two photoperiods at the 5% significance level.

On a fresh weight basis, the Ca and Mg contents were significantly higher under the 24-h photoperiod at the 0.1% and 1% significance levels, respectively. However, the Mn content was significantly lower under the 24-h photoperiod at the 5% significance level. The P, K, Na, Fe, and Zn contents were not significantly different between the two photoperiods at the 5% significance level.

Ascorbic acid content

On a dry weight basis, the ascorbic acid content tended to be higher under the 24-h photoperiod compared with under the 12-h photoperiod, but there was no significant difference at the 5% significance level between the two photoperiods (Fig. 6). On a fresh weight basis, the ascorbic acid content was 1.8-times higher under the 24-h photoperiod, which was significant at the 5% level.

Chlorogenic acid and total polyphenol contents

The chlorogenic acid content was 2.5-times higher on a dry weight basis and 3.1-times higher on a fresh weight basis under the 24-h photoperiod than under the 12-h photoperiod, and these differences were significant at the 5% and 1% levels, respectively (Fig. 6). Similarly, the total polyphenol content was 1.5- and 1.8-times higher on dry and fresh weight bases, respectively, under the 24-h photoperiod, and these differences were significant at the 1% and 0.1% levels, respectively. There was a positive correlation between chlorogenic acid and total polyphenol contents, with a correlation coefficient of 0.48 on a dry weight basis, whereas the correlation coefficient was 0.64 on a fresh weight basis, indicating a stronger correlation (Fig. 7).

Table 2 - Effects of photoperiod on the stem development of Japanese mugwort plants

Photoperiod (h)	No. of branches	Main stem		
		Length (cm)	No. of nodes	Mean of internode length (cm)
12	15.0	20.65	23.25	0.9
24	22.0	33.10	28.45	1.1
Significance [z]	***	**	***	**

[z] Statistical significances between the means of two photoperiods were determined using Student's t-test (n = 10). **, p < 0.01; ***, p<0.001.

Table 3 - Effects of photoperiod on the mineral contents of Japanese mugwort leaves

Photoperiod (h)	Mineral content on a dry weight basis							
	P (mg g DW^{-1})	K (mg g DW^{-1})	Ca (mg g DW^{-1})	Mg (mg g DW^{-1})	Na (mg g DW^{-1})	Fe (µg g DW^{-1})	Mn (µg g DW^{-1})	Zn (µg g DW^{-1})
12	13.2	48.7	11.5	3.2	0.3	152.2	325.8	60.7
24	10.8	39.1	11.8	3.1	0.3	126.2	211.7	44.3
Significance[z]	NS	**	NS	NS	NS	***	***	**

Photoperiod (h)	Mineral content on a fresh weight basis (mg g FW^{-1})							
	P	K	Ca	Mg	Na	Fe	Mn	Zn
12	138.0	511.9	121.2	33.4	3.1	1.6	3.5	0.6
24	140.9	510.3	154.3	41.0	3.5	1.7	2.8	0.6
Significance[z]	NS	NS	***	**	NS	NS	*	NS

Statistical significances between the means of two photoperiods were determined using Student's t-test (n = 10). NS= not significant; * p < 0.05; ** p < 0.01; *** p < 0.001.

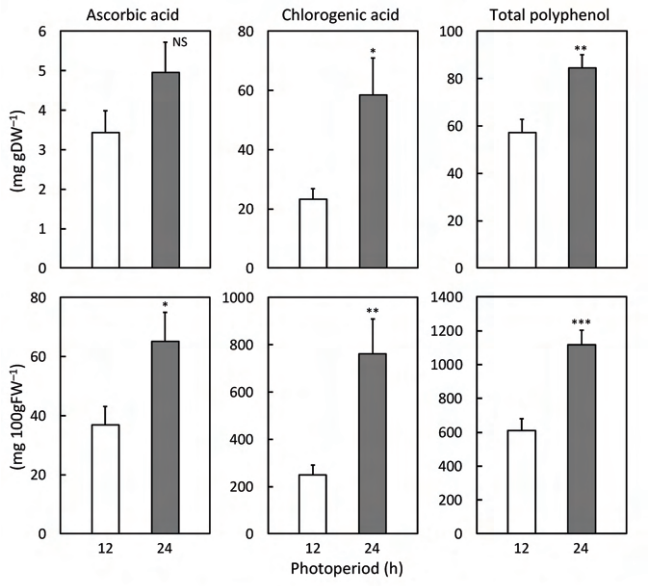

Fig. 6 - Effects of photoperiod on ascorbic acid, chlorogenic acid, and total polyphenol contents in Japanese mugwort leaves. Values represent means ± SEs (n = 10). Statistical significances between the two photoperiods were determined using Student's t-test. NS, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

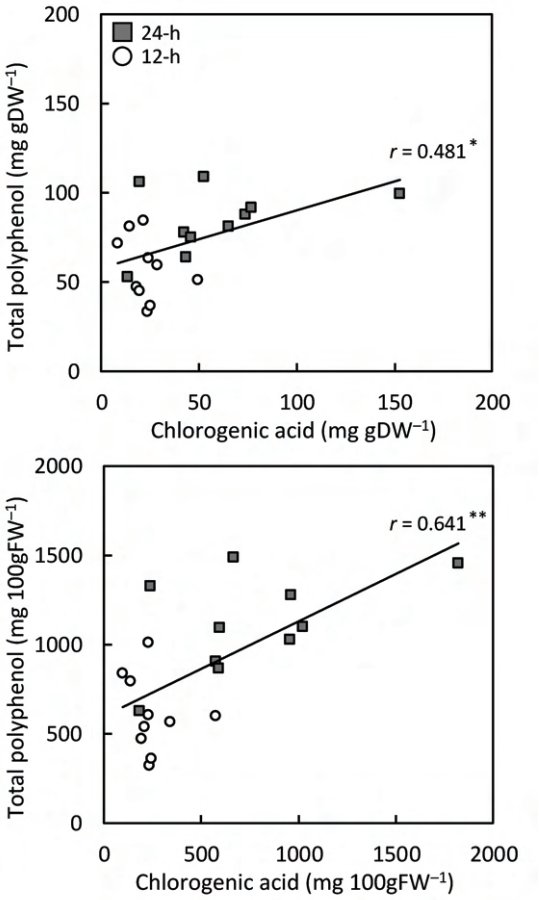

Fig. 7 - Correlations between chlorogenic acid and total polyphenol contents in the leaves of Japanese mugwort plants grown under 12-h (open circles) and 24-h (closed squares) photoperiods. * and ** indicate significant correlations as determined by Pearson's test at p < 0.05 and p < 0.01, respectively (n = 20).

4. Discussion and Conclusions

Change in nutrient solution pH

We reported previously (Hata and Kawamura, 2021) that when growing 'Ibuki-yomogi' plants hydroponically in a greenhouse for 4 weeks at differ-

ent nutrient solution concentrations, the nutrient solution pH increases as the nutrient solution concentration decreases and that after lowering the latter to 25% of the standard, the nutrient solution pH reached 8.1. Similarly, in the present study, the nutrient solution pH increased, even when the nutrient solutions' initial pH was adjusted to 6.0, indicating that the nutrient solutions' pH tended to increase even under artificial light sources, regardless of the photoperiod, by lowering the concentration to 25% of the standard.

When anion uptake is dominant, the rhizosphere pH increases as a result of OH^- or HCO_3^- release from the roots to maintain the cellular charge balance, and when cation uptake is dominant, H^+ is similarly released from the roots and the rhizosphere pH decreases (Hinsinger *et al*., 2003; Fageria, 2012). In particular, because NO_3^- and NH_4^+ account for approximately 70% of the cations and anions absorbed by plants, the form of nitrogen application has a significant effect on rhizosphere pH (nutrient solution pH). Furthermore, Zheng *et al*. (2004, 2010) reported that the medium pH increases when the applied nutrient solution concentration is lowered from 100% to 25% in the pot cultivation of rose and gerbera, indicating that the rhizosphere pH (nutrient solution pH) may not decrease, but rather increase, owing to the lack of NH_4^+ at a low nutrient solution concentration. Because the total nitrogen content in the Enshi formula nutrient solution used in the present study consisted of 92.5% NO_3^- and 7.5% NH_4^+, the plants absorbed less NH_4^+ as the nutrient solution concentration decreased, which may have caused the nutrient solution pH to increase, rather than decrease, during the growing period.

Masuda *et al*. (2001) reported that when pepper plants are cultivated hydroponically with fluorescent lamps under a 24-h photoperiod, the pH of the recirculating nutrient solution rapidly increases immediately after planting. In the present study, similarly, the nutrient solution pH at 2 weeks after planting or 1 week after nutrient solution renewal increased more under the 24-h photoperiod compared with the 12-h photoperiod. On the other hand, Hata and Xu (2020 a) reported that when leaf lettuce is grown hydroponically using a nutrient solution containing NH_4^+ as the nitrogen source, the degree of decrease in nutrient solution pH is greater under the 24-h photoperiod than under the 12-h photoperiod, suggesting that a faster the growth rate, the pH is more likely to decrease. Thus, the faster the growth rate, the greater the change in the pH of the nutrient solution in proportion to the amount of nitrogen absorbed. Consequently, greater growth rates of the mugwort plants under the 24-h photoperiod than under the 12-h photoperiod makes the pH of the nutrient solution more likely to increase, when grown using a low-concentration of nutrient solution.

Plant growths

For plants that are capable of cultivation under longer photoperiods, the production cost per plant in a fully artificial light-type plant factory decreases as the photoperiod increases, and a 24-h photoperiod is desirable (Takatsuji, 2012). Plants capable of longer photoperiods are highly tolerant of the continuous light injury that occurs under a 24-h photoperiod, and their growth is greatly accelerated. The occurrence of a marked level of continuous light injury in Asteraceae plants has not been reported to date, and maximum plant growth rates have been reported under 24-h photoperiods in lettuce and garland chrysanthemum (Hata *et al*., 2011 a, b). This was also the case for the Japanese mugwort plants used in the present study. Continuous light-induced chlorosis did not occur in newly developed leaves, the leaf color darkened under the 24-h photoperiod, and the dry weights of leaves, stems, and roots at the end of cultivation were nearly two-fold greater under the 24-h photoperiod than under the 12-h photoperiod. Thus, like lettuce and garland chrysanthemum, Japanese mugwort, which is a member of the Asteraceae family, is not susceptible to continuous light injury. Thus, a 24-h photoperiod could be used to increase the leaf yield and productivity of Japanese mugwort in a fully artificial light-type plant factory.

Stem elongation in plants is inhibited by greater red to far-red light ratios (R/FRs), whereas lower R/FRs may promote plant stem elongation owing to greater internode elongation (Demotes-Mainard *et al*., 2016; Ballaré and Pierik, 2017). White fluorescent light has a higher R/FR ratio than sunlight (6.5–9.6 and 1.0, respectively) (Hamamoto and Yamazaki, 2013), and internode elongation is likely to be suppressed in a plant factory environment that uses such fluorescent lighting. In fact, in our previous report using the same 'Ibuki-yomogi' seeds and hydroponic cultivation method, the average internode length of individuals grown in a glasshouse was 2.3 cm (Hata and Kawamura, 2021), whereas the average internode length of individuals grown in an artificial growth room, as in the present study, was 0.9-1.1 cm. The

internode shortening under fluorescent light is advantageous for producing young leaves of Japanese mugwort plants because of the low plant heights in the multi-shelf cultivation system used in plant factories. In addition, the number of branches was significantly higher under the 24-h photoperiod than under the 12-h photoperiod, suggesting that cultivation under the former is advantageous for increasing the number of harvested stems.

Inorganic component contents

The carbon content increases, whereas other essential inorganic element contents generally decrease, in many plant species when grown at greater than atmospheric CO_2 concentrations (Loladze, 2014; Soares *et al*., 2019). The factors responsible for the decrease in these inorganic elements include (1) a decreased transpiration rate leading to a lowered absorption, and (2) an increased carbon content which results in a reduced element relative content (dilution by carbohydrates). Although there have been limited studies on the effects of a 24-h photoperiod on the inorganic component contents in plants, Hata and Xu (2020 b) found that when leaf lettuce is grown under a 24-h photoperiod, the leaf carbon content increases more compared with under a 12-h photoperiod, whereas many inorganic component contents decrease, suggesting that reactions similar to those under high CO_2 conditions occur under a 24-h photoperiod. The K, Fe, Mn, and Zn contents per dry weight of Japanese mugwort in the present study were also significantly lower under the 24-h photoperiod compared with the 12-h photoperiod, suggesting that there may be a number of plant species in which the inorganic component contents tend to decrease under a 24-h photoperiod. As in leaf lettuce (Hata and Xu, 2020 a, b), no clear nutrient deficiency symptoms associated with decreased inorganic component contents were observed in Japanese mugwort in the present study under a 24-h photoperiod, but lower-leaf browning was observed in some individuals, suggesting that the potassium concentration in the culture medium requires optimization.

Ascorbic acid content

Ascorbic acid in plants is synthesized through the D-Man/L-Gal pathway, in which D-fructose, a photosynthetic product, is used as a metabolic intermediate to synthesize D-mannose and L-galactose (Venkatesh and Park, 2014). In leaf lettuce, the ascorbic acid content per fresh weight increases 1.3-fold when grown under a 24-h photoperiod compared with under a 16-h photoperiod owing to an increase in the activity of L-galactono-1,4-lactone dehydrogenase, an enzyme that converts L-galactono-1,4-lactone, an ascorbic acid precursor, to ascorbic acid (Zha *et al*., 2019). In the present study, the ascorbic acid content per fresh weight was 1.8-times higher under the 24-h photoperiod than under the 12-h photoperiod, which was consistent with previous results. This is suggested that a 24-h photoperiod may be used effectively in the production of crops having enhanced ascorbic acid contents, unless the target plants develop continuous light injuries. The addition of ascorbic acid suppresses the degradation of polyphenols, such as chlorogenic acid, during apple juice processing (Kolniak-Ostek *et al*., 2013), indicating that the increased ascorbic acid content in Japanese mugwort leaves may contribute to the increased stability of polyphenols, such as chlorogenic acid, during utilization.

Chlorogenic acid and total polyphenol contents

Hata and Xu (2020 b) reported that the chlorogenic acid and total polyphenol contents per dry weight increased by 1.5 to 4.2 times and 1.1 to 1.2 times, respectively, in leaf lettuce grown under a 24-h photoperiod compared with under a 12-h photoperiod. Furthermore, the differences between the two photoperiods widened in the chlorogenic acid and total polyphenol contents per fresh weight because the dry matter ratio increased more under the 24-h photoperiod than under the 12-h photoperiod. In the present study, the chlorogenic acid content was 2.5- and 3.1-times higher per dry and fresh weights, respectively, and the total polyphenol content was 1.5- and 1.8-times higher per dry and fresh weights in Japanese mugwort under a 24-h photoperiod compared with a 12-h photoperiod. These results were similar to those previously reported for leaf lettuce (Hata and Xu, 2020 b), and they suggest that a 24-h photoperiod may be effectively used for the production of crops with high polyphenol contents, such as chlorogenic acid, unless target plants develop continuous light injuries.

We reported previously (Hata and Kawamura, 2021) that when 'Ibuki-yomogi' plants are grown hydroponically in a glasshouse, the chlorogenic acid and total polyphenol contents of the leaves increase

as the nutrient solution concentration decreases to 25% of the standard. There were positive correlations (r = 0.45) between chlorogenic acid and total polyphenol contents, both per dry weight and per fresh weight bases. In the present study, positive correlations between chlorogenic acid and total polyphenol contents (r = 0.48 for content per dry weight and r = 0.64 for content per fresh weight) were also observed, suggesting that the increase in the former largely contributed to the increase the latter under a 24-h photoperiod. In addition, high anthocyanin pigment accumulations are often observed in some plant species under a 24-h photoperiod (Hata *et al*., 2012 b), and here, we observed some individuals accumulating anthocyanin pigments in the main stems under the 24-h photoperiod. This suggests that anthocyanin synthesis is also enhanced in Japanese mugwort under a 24-h photoperiod and that increases in some flavonoid compounds may also contribute to the increase in the total polyphenol content.

These results indicate that it is possible to grow Japanese mugwort hydroponically under a 24-h photoperiod and plant factory conditions in a nutrient solution having a concentration as low as 25% of the standard. In addition, under the 24-h photoperiod, plant growth was greatly accelerated and chlorogenic acid, a useful secondary metabolite, as well as ascorbic acid, contents increased, suggesting that a 24-h photoperiod is highly beneficial for Japanese mugwort production in a fully artificial light-type plant factory. However, because the pH of the nutrient solution fluctuated drastically during the cultivation period, it is necessary to investigate separately the composition of the nutrient solution suitable for a 24-h cultivation photoperiod.

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