



저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:



저작자표시. 귀하는 원저작자를 표시하여야 합니다.



비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.



변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 [이용허락규약\(Legal Code\)](#)을 이해하기 쉽게 요약한 것입니다.

[Disclaimer](#)

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

**Effects of Seasonal Light Variation and Artificial
Light Treatments on Growth and Flavonoid
Production of *Artemisia princeps* Cultivated in
Greenhouses**

계절적 광 변화 및 인공 조명 처리가 온실 재배
약쑥(*Artemisia princeps*)의 생장 및 플라보노이드
생산에 미치는 영향

BY
ZEESOO HAN

FEBRUARY, 2019

MAJOR IN HORTICULTURAL SCIENCE AND BIOTECHNOLOGY
DEPARTMENT OF PLANT SCIENCE
THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

**Effects of Seasonal Light Variation and Artificial Light Treatments
on Growth and Flavonoid Production of *Artemisia princeps*
Cultivated in Greenhouses**

Zeesoo Han

Department of Plant Science
The Graduate School of Seoul National University

ABSTRACT

Artemisia princeps (Ganghwa wormwood) is a medicinal plant with two major flavonoids, eupatilin and jaceosidin, which have gastritis and peptic ulcers treatment properties. Until now, the plants are cultivated in the fields which provides one cultivation period, and produces unstable flavonoid contents by environmental changes. The objective of this study was to analyze the effects of seasonal light variation and artificial light treatments on growth and flavonoid production of *A. princeps* grown under greenhouse conditions for year-round production. From April 2016 to April 2017, nine sets of the plants were cultivated and harvested under natural seasonal light conditions in greenhouses. From September 2016 to January 2017, four additional artificial light treatments were applied for two sets of the plants: supplemental light, night interruption, low light, and low light with night interruption. The

plants grown under natural light condition in greenhouses were used as a control. After harvest, plant growth was measured, and the contents of eupatilin and jaceosidin were determined. The plants had the highest biomass when the accumulated radiation and duration were highest. Plant growth and flavonoid production were significantly associated with accumulated radiation and light duration. Supplemental light and night interruption treatments resulted in significantly higher biomass and flavonoid production. For consistent biomass and flavonoid production of *A. princeps*, night interruption treatment is suggested in greenhouse cultivation during low irradiation and short days (less than 13 h).

Key words: *Artemisia princeps*, Eupatilin, Greenhouse cultivation, Jaceosidin, Light duration, Light intensity, Year-round production

Student number: 2015-23014

CONTENTS

ABSTRACT	i
CONTENTS	iii
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
LITERATURE REVIEW	4
Pharmaceutical uses of <i>A. princeps</i>	4
Flavonoids and environment conditions	4
Medicinal plant cultivation in controlled environments	5
MATERIALS AND METHODS	6
RESULTS AND DISCUSSION	13
CONCLUSIONS	29
LITERATURE CITED	30
ABSTRACT IN KOREAN	36

LIST OF TABLES

Table 1. Experiment schedules of nine different seasonal cultivation periods and applied artificial light treatments.....	7
Table 2. Accumulated and mean light conditions of natural sunlight (NL), supplemental light (SL), night interruption (NI), low light (LL), and low light with night interruption (LN) of Exps. 6 and 7.....	10
Table 3. Growth characteristics of <i>A. princeps</i> cultivated under natural sunlight (NL) during nine different seasonal cultivation periods.....	14

LIST OF FIGURES

Fig. 1. HPLC chromatogram and chemical structures of eupatilin and jaceosidin of <i>A. princeps</i>	12
Fig. 2. Fresh (A) and dry weights (B) of whole plant and leaf of <i>A. princeps</i> cultivated during nine different seasonal cultivation periods.....	15
Fig. 3. Accumulated light duration (A) and radiation (B) of nine different seasonal cultivation periods.....	16
Fig. 4. Eupatilin content and total eupatilin production (eupatilin content × leaf dry weight) in <i>A. princeps</i> cultivated during nine different seasonal cultivation periods.....	17
Fig. 5. Jaceosidin content and total jaceosidin production (jaceosidin content × leaf dry weight) in <i>A. princeps</i> cultivated during nine different seasonal cultivation periods.....	18
Fig. 6. Relationships of leaf dry weights of <i>A. princeps</i> with accumulated radiation (A) and light duration (B).....	19
Fig. 7. Relationships of total eupatilin production in <i>A. princeps</i> with accumulated radiation (A) and light duration (B).....	21
Fig. 8. Relationships of total jaceosidin production in <i>A. princeps</i> with accumulated radiation (A) and light duration (B).....	23

Fig. 9. Eupatilin content (A) and total eupatilin production (B) in *A. princeps* of Exps. 6 and 7 25

Fig. 10. Jaceosidin content (A) and total jaceosidin production (B) in *A. princeps* of Exps. 6 and 7 27

INTRODUCTION

Artemisia is a perennial herb distributed in the Northern hemisphere and one of the largest genera of the Asteraceae or Compositae family. *Artemisia* species have been used in traditional herbal medicine to treat microbial infections, inflammatory diseases, gastric ulcers, and circulatory disorders (Ryu et al., 2005). In *A. princeps* (Ganghwa wormwood), two flavonoids, eupatilin (5,7-dihydroxy-6,3',4'-trimethoxy flavone) and jaceosidin (5,7,4'-trihydroxy-6,3'-dimethoxy flavone), are potent anti-ulcer compounds (Lee et al., 2007), and are used to treat gastric ulcers.

However, the growth of the plants with eupatilin and jaceosidin contents is highly dependent on the environmental factors at the cultivation site. For example, from the plants cultivated in 48 different geographical locations in South Korea, eupatilin contents varied with an average of 43.8 mg per 100 g of leaves, and 12.8 mg for jaceosidin contents (Ryu, 2008). In different studies of *A. princeps* cultivation in different geographical locations, the flavonoid contents also highly varied from 168.5 to 217.3 mg in eupatilin, and 28.6 to 38.6 mg in jaceosidin contents (Ahn et al., 2012; Ryu et al., 2005). These analyses suggest that the eupatilin and jaceosidin contents can be stabilized with cultivation in controlled environments by year-round consistent production (Fonseca et al., 2006).

Artemisia cultivation in controlled environments has been reported to

increase the production of target pharmaceutical raw materials. Artemetin, a flavone from *A. absinthium* (absinth wormwood) which has acaricidal, insecticidal, and repellent effects, was significantly higher in greenhouse cultivation than in field cultivation (Gonzalez-Coloma et al., 2012). *A. vulgaris* (common wormwood) cultivated in a greenhouse with a hydroponics system produced 2.95-times higher yield of biomass than those cultivated in the field (Dorais et al., 2001). To use *A. princeps* as a pharmaceutical raw material for industrial production in the pharmaceutical industry, a controlled environment can provide more stable and higher yield than field condition. An additional advantage of production of medicinal plants in controlled environments is that the plants could be free from biotic and abiotic factors, allowing uniform and consistent growth and production of secondary metabolites (Zobayed and Saxena, 2004).

Among various controlled environments, a solar greenhouse is effective for the cultivation by providing solar radiation as *A. princeps* characteristic of a full sun plant. However, natural sunlight in greenhouses does not produce consistent light intensities and durations because of seasonal light changes. Plants will have fluctuated growth and flavonoid production. The objectives of this study were to analyze the effects of seasonal light variation and light treatments on the growth and flavonoid production of *A.princeps* cultivated under greenhouse conditions for year-

round consistent biomass and flavonoid production.

LITERATURE REVIEW

Pharmaceutical uses of *A. princeps*

A. princeps, native to Ganghwa island, South Korea, contains anti-inflammatory, cardiogenic, and antitussive efficacies (Bang et al., 2008), and has anti-bacterial pharmacological action (Cho and Chiang, 2001). Active ingredients isolated from *A. princeps* contain flavonoids of eupatilin, jaceosidin, and apigenin (Ryu et al., 2005), and sterols of β -sitosterol, ergosterol, peroxide, daucosterol, and stigmasterol (Bang et al., 2006). Since eupatilin has strong inhibitory effects on gastric ulcer and the growth of cancer cells, it has been used as a pharmaceutical raw material as a natural product (Kim et al., 2004; Oh et al., 1997; Ryu et al., 2005).

Flavonoids and environment conditions

Plants have developed mechanisms, the synthesis of secondary metabolites including flavonoids, against unfavorable environmental conditions (Mierziak et al., 2014). Flavonoids diminish the effects caused by reactive oxygen species during the presence of oxidative stress (Treutter, 2005). Among various environmental factors, light intensity, photoperiod, and temperature influence the biosynthesis of flavonoids (Jaakola and Hohtola, 2010), as Davik et al. (2006) reported that the longer photoperiod and lower night temperatures of northern latitudes

increased the production of aromatic compounds compared to the plants in southern latitudes.

Medicinal plant cultivation in controlled environments

Zobayed et al. (2005) reported that in comparison to traditional field- or wild-harvest medicinal plants, controlled environments are an alternative way for medicinal plant cultivation by its protection and control of biotic and abiotic contamination, alteration with misidentified plant species or weeds, variability in medicinal plant products, and genetic variability.

Increased production of target medicinal compound is possible by environmental control as Ferreira et al. (1995) cultivated *A. annua* (sweet wormwood) in a greenhouse with the objective of increasing artemisinin production by controlling photoperiod for flowering, as artemisinin content was 25% higher in inflorescences than in leaves. Light intensity control in a growth chamber suggested an optimum light intensity of $30 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for highest biomass and total flavonoids production of *Anoectochilus* plants (Ma et al., 2010).

MATERIALS AND METHODS

Plant material and growth conditions

The experiments were conducted in a Venlo-type glasshouse located in the experimental farm of Seoul National University, Suwon, Korea (latitude, 37.3°N; longitude, 127.0°E). The air temperature and relative humidity were maintained at $30.9 \pm 3.70^\circ\text{C}$ (day) / $22.2 \pm 3.30^\circ\text{C}$ (night) and $52.0 \pm 18.4\%$, respectively.

Five separate mother stock roots of *A. princeps* in 10 cm long were planted into a pot (36 · 48 · 15 cm, L · W · H) with growth medium (Barokuh 2, Seoul Bio, Eumsung, Korea). After when the plant height was at least 30 cm, 5 cm long unrooted cuttings including one leaf and a node were propagated into a 50-cell plug tray (26.5 · 53 · 5.5 cm, L · W · H). After 6 weeks of the seedling stage, each seedling was transplanted into a pot (Φ 12 · H 11 cm) in a plant density of 72 plants · m⁻². After transplanting, plants were cultivated for 8 weeks. The leaves were collected for the determination of eupatilin and jaceosidin production, as only leaves from *A. princeps* are known to contain these flavonoids (Kim et al., 2013). Leaf samples were harvested nine times between July 2016 and April 2017 for the analyses of the flavonoids production, and additionally eight times for experiments with artificial light treatments (Exps. 6, 7; Table 1).

Nutrient solutions consisted of 230 mg · L⁻¹ Ca(NO₃)₂ · 4H₂O, 10 mg · L⁻¹ NH₄NO₃, 1.74 mg · L⁻¹ Fe-EDTA, 106 mg · L⁻¹ KNO₃, 86 mg · L⁻¹ MgSO₄ ·

Table 1. Experiment schedules of nine different seasonal cultivation periods and applied artificial light treatments.

Exp. No.	Cultivation period (yyyy-mm-dd)			Light treatment
	Propagation	Transplantation	Harvest	
1	2016-04-05	2016-05-16	2016-07-12	NL ^z
2	2016-05-10	2016-06-21	2016-08-16	NL
3	2016-05-31	2016-07-12	2016-09-06	NL
4	2016-06-28	2016-08-09	2016-10-05	NL
5	2016-07-26	2016-09-07	2016-11-01	NL
6	2016-09-06	2016-10-19	2016-12-15	NL, SL, LL, NI, LN
7	2016-09-23	2016-11-06	2017-01-01	NL, SL, LL, NI, LN
8	2016-12-02	2017-01-13	2017-03-09	NL
9	2017-01-01	2017-02-12	2017-04-07	NL

^zNL, natural light; SL, supplemental light; LL, low light; NI, night interruption; LN, low light and night interruption during the period when light duration was less than 13 h.

7H₂O, 34.4 mg · L⁻¹ KH₂PO₄, 0.56 mg · L⁻¹ H₃BO₃, 0.48 mg · L⁻¹ ZnSO₄ · 7H₂O, 0.05 mg · L⁻¹ MnSO₄ · 5H₂O, 0.05 mg · L⁻¹ CuSO₄ · 5H₂O, and 0.02 mg · L⁻¹ NaMoO₄ · 2H₂O. Solutions were subirrigated 15 min per day during the 6 weeks of seedling cultivation and 8 weeks of cultivation after transplanting. The electrical conductivity and pH were maintained at 1.5 dS · m⁻¹ and 6.5, respectively.

Light conditions and treatments

Light treatments were scheduled differently throughout the year based on the light duration of the season (Table 1) to determine the effects of light quantity on plant growth and flavonoid production. From April 2016 to April 2017, the plants were propagated every month and cultivated for 14 weeks under natural light inside the greenhouse. For Exps. 6 and 7, during the season when the light duration was less than 13 h, four additional artificial light treatments (supplemental lighting, night interruption, low lighting, and low lighting with night interruption) were applied to determine the effects of light intensity and duration on growth and flavonoid production. The eupatilin and jaceosidin contents are highest during the vegetative stage and decrease after physiological maturing (Ryu, 2008), and as *Artemisia* is a short-day plant with a critical photoperiod of 13 h (Ferreira et al., 1995; Jelodar et al., 2014), 13 h was used as a standard for light treatments. For the supplemental light

treatment, a 250-W metal halide lamp ($80 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) was installed 2 m above the pot and was on for 13 h (06:00-19:00). For low light treatment, a 75% shading screen was installed 2 m above the pot. A 15-W cool-white fluorescent lamp ($10 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) was used for 4 h (22:00-02:00) for night interruption treatment. Light intensities (Table 2) were measured on the surface of the pot with a light meter (LI-250A; LI-COR, Lincoln, NE, USA).

Plant growth measurements

Five plants per experiments and artificial light treatments were harvested 57 days after transplanting for destructive measurements and analyses. Growth characteristics of plant height, number of nodes, chlorophyll content (SPAD value), stem diameter, leaf area, and fresh and dry weights of whole plant and leaf were measured. Plant height was measured from the soil surface to the top of the main stem. Stem diameter was measured on the main stem beneath the first node of the main stem with a digital vernier caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan). The chlorophyll content was measured on five fully developed leaves with a chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan). The stem and leaf fresh and dry weights were measured with a digital scale (MW2N-300, CAS, Seoul, Korea). Dry weights were measured after oven-drying at 80°C for 72 h.

Table 2. Accumulated and mean light conditions of natural sunlight (NL), supplemental light (SL), night interruption (NI), low light (LL), and low light with night interruption (LN) of Exps. 6 and 7.

Light treatment	Accumulated radiation (MJ·m ⁻²) ^z	Mean daily radiation (MJ·m ⁻²) ^y	Accumulated light duration (h) ^x	Mean light duration (h) ^w
Exp. 6 (2016-09-06 to 2016-12-15)				
NL	240.0	4.14	592.9	10.2
SL	268.4	4.63	754.0	13.0
NI	241.1	4.16	824.9	14.2
LL	60.0	1.03	592.9	10.2
LN	61.0	1.05	824.9	14.2
Exp. 7 (2016-09-23 to 2016-01-01)				
NL	219.0	3.84	564.4	9.90
SL	246.9	4.33	741.0	13.0
NI	220.1	3.86	792.4	13.9
LL	54.7	0.96	564.4	9.90
LN	55.8	0.98	792.4	13.9

^zAccumulated radiation = cumulative radiation during the cultivation period.

^yMean daily radiation = accumulated radiation divided by cultivation days.

^xAccumulated light duration = cumulative light duration during the cultivation period.

^wMean light duration = accumulated light duration divided by cultivation days.

Flavonoid determination

The harvested leaves were dried in an oven at 80°C for 72 h. The dried leaves were ground in a blender for the determination of eupatilin and jaceosidin. Then, 1 g (dry weight) of leaves from each of the five plants was collected from each of the experiments and light treatments. On the HPLC chromatogram of flavonoids, eupatilin and jaceosidin were detected in *A. princeps* (Fig. 1). For the HPLC analyses, 10 mL of 95% (v/v) ethanol was added to the leaves and ultrasonicated for 2 h. The HPLC column was a Luna (II) C18 reverse phase column (Φ 4.6 · H 250 mm) (Phenomenex, Macclesfield, UK), used at a column temperature of 35°C, flow rate of 1.0 mL · min⁻¹, sample volume of 10 μ L, and run time of 40 min. The mobile phase was eluted with acetonitrile (ACN) and water with 0.1% trifluoroacetic acid (TFA) at the following gradient: 0 min (ACN:0.1% TFA water = 30:70), 18 min (42:68), 25 min (60:40), 32 min (95:5), and 40 min (30:70).

Statistical analysis

Statistical significance was determined using a one-way analysis of variance (ANOVA) and mean separation between experiments and light treatments using Tukey's test at $P < 0.05$ with SPSS, version 21 (IBM, Armonk, NY, USA).

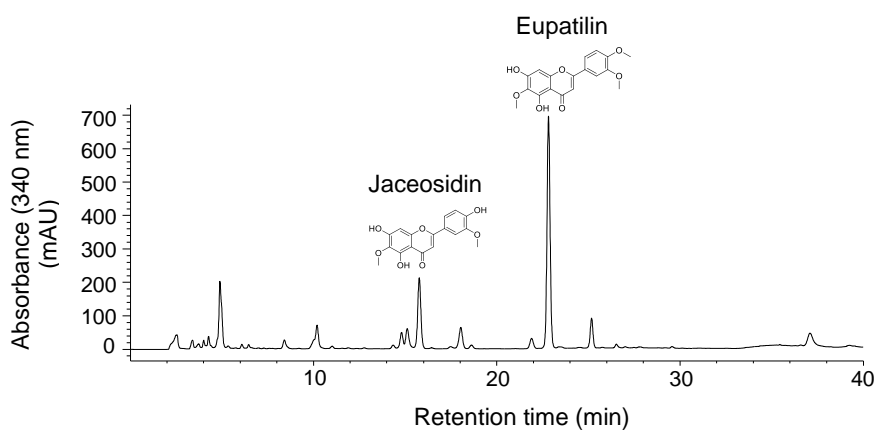


Fig. 1. HPLC chromatogram and chemical structures of eupatilin and jaceosidin of *A. princeps*.

RESULTS AND DISCUSSION

Plant growth and flavonoid production under seasonal natural light conditions

Among the different cultivation periods, plant growth was greatest in Exp. 1, followed by Exp. 2 (Table 3). Plant total dry weight was 1.95-times greater, and leaf dry weight was 1.73-times greater in Exp. 1 than in Exp. 2 (Fig. 2). The difference in growth between Exp. 1 and Exp. 2 was related to the difference in accumulated radiation and light duration during the cultivation periods (Figs. 3A, 3B). Eupatilin content was highest in Exp. 9, followed by Exp. 1 (Fig. 4A). However, as the leaf dry weight was greater in Exp. 1 than in Exp. 9 (Fig. 2B), total eupatilin production was 2.56-times higher in Exp. 1 than in Exp. 9 (Fig. 4B). Similarly, even though the jaceosidin content of Exp. 2 was higher than that of Exp.1 (Fig. 5A), total jaceosidin production was higher in Exp.1 than in Exp. 2 (Fig. 5B), indicating the plant biomass highly contributed to the total flavonoids production during the cultivation period.

Plant growth was highly related to the accumulated radiation and light duration (Table 3, Figs. 3A, 3B), as the increase in accumulated radiation and light duration increased the dry weight of the plants (Figs. 6A, 6B). Light intensity is one of the key environmental factors for plant growth. As the accumulated radiation and light duration decreased, growth and biomass accumulation of sun plants also decreased (Höft et al., 1996).

Table 3. Growth characteristics of *A. princeps* cultivated under natural sunlight (NL) during nine different seasonal cultivation periods. Refer to Table 1 for Exp. No. schedules.

Exp. No.	Plant height (cm)	No. of nodes	Stem diameter (mm)	SPAD value	Leaf area (mm ²)
1	135.0 ± 8.54a ^{z,y}	56.0 ± 3.58ab	6.47 ± 0.30a	53.6 ± 3.94b	3010.5 ± 169.1a
2	120.0 ± 5.31a	48.8 ± 1.99bc	4.16 ± 0.29bc	39.8 ± 1.64cd	1557.8 ± 126.4b
3	131.4 ± 3.31a	65.0 ± 1.59a	4.71 ± 0.29b	47.9 ± 1.43bc	1029.3 ± 70.3bcd
4	122.3 ± 4.66a	52.0 ± 1.90ab	3.72 ± 0.31bcd	40.4 ± 1.53cd	927.7 ± 41.0cd
5	82.2 ± 3.65b	47.0 ± 0.63bcd	3.33 ± 0.24bcd	39.9 ± 2.18cd	903.7 ± 37.0cd
6	55.8 ± 3.91b	32.0 ± 2.06d	2.37 ± 0.18d	29.2 ± 0.94d	489.9 ± 47.0d
7	57.2 ± 7.93b	31.6 ± 1.66d	2.36 ± 0.09d	34.2 ± 0.65d	416.7 ± 33.2d
8	51.1 ± 2.46b	33.8 ± 0.89cd	2.64 ± 0.03d	36.9 ± 0.04cd	640.6 ± 1.87cd
9	75.7 ± 5.23b	44.0 ± 1.91cd	2.69 ± 0.28cd	33.0 ± 1.55d	588.5 ± 70.5cd

^zMean ± standard error (n = 5)

^yDifferent letters represent significant differences by Tukey's test at $P < 0.05$.

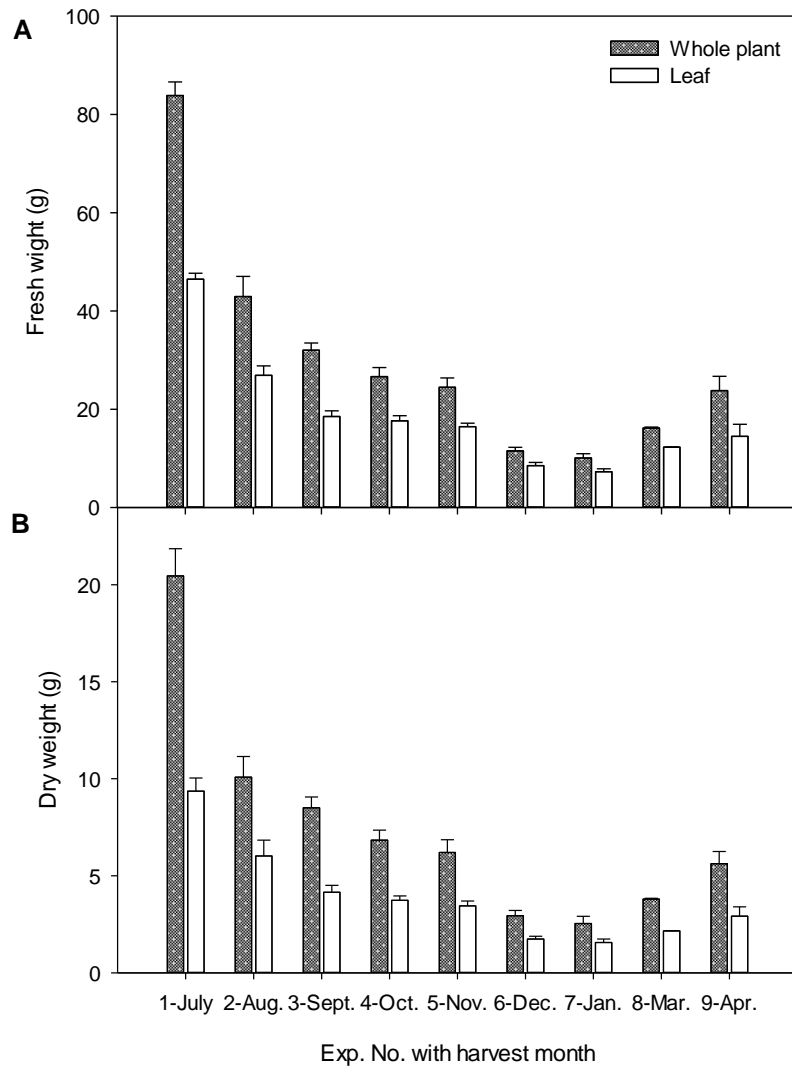


Fig. 2. Fresh (A) and dry weights (B) of whole plant and leaf of *A. princeps* cultivated during nine different seasonal cultivation periods. Vertical bars represent standard errors of the means (n = 5).

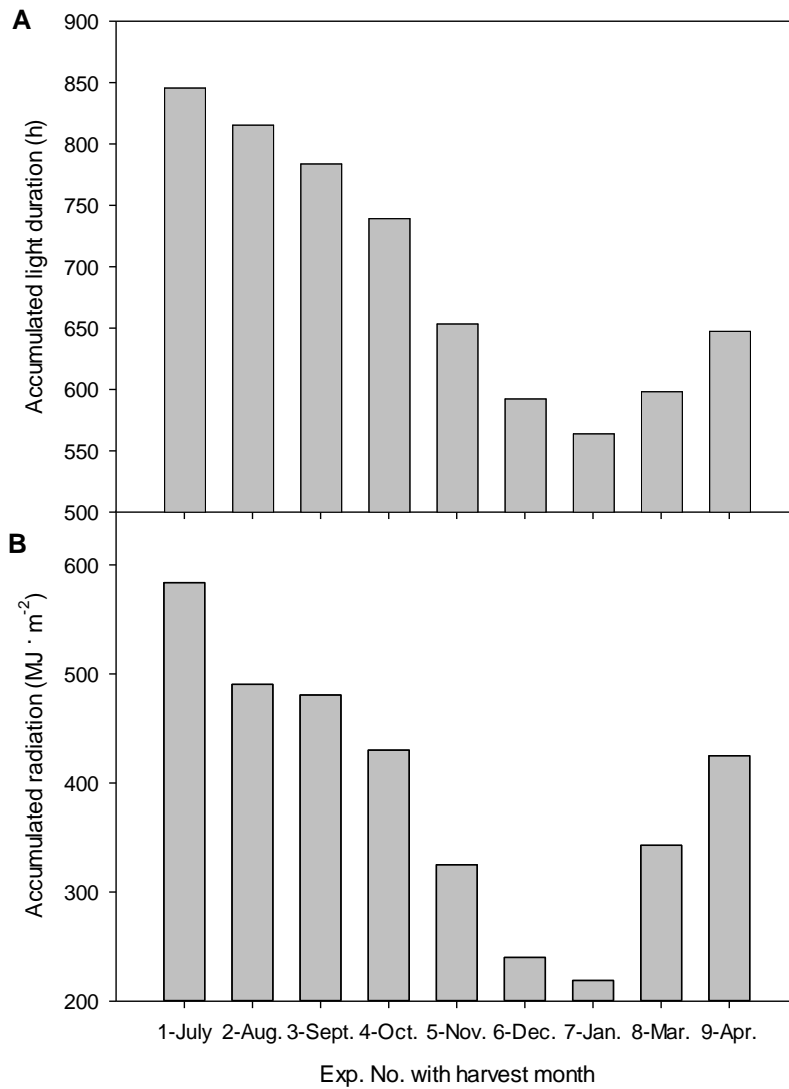


Fig. 3. Accumulated light duration (A) and radiation (B) of nine different seasonal cultivation periods.

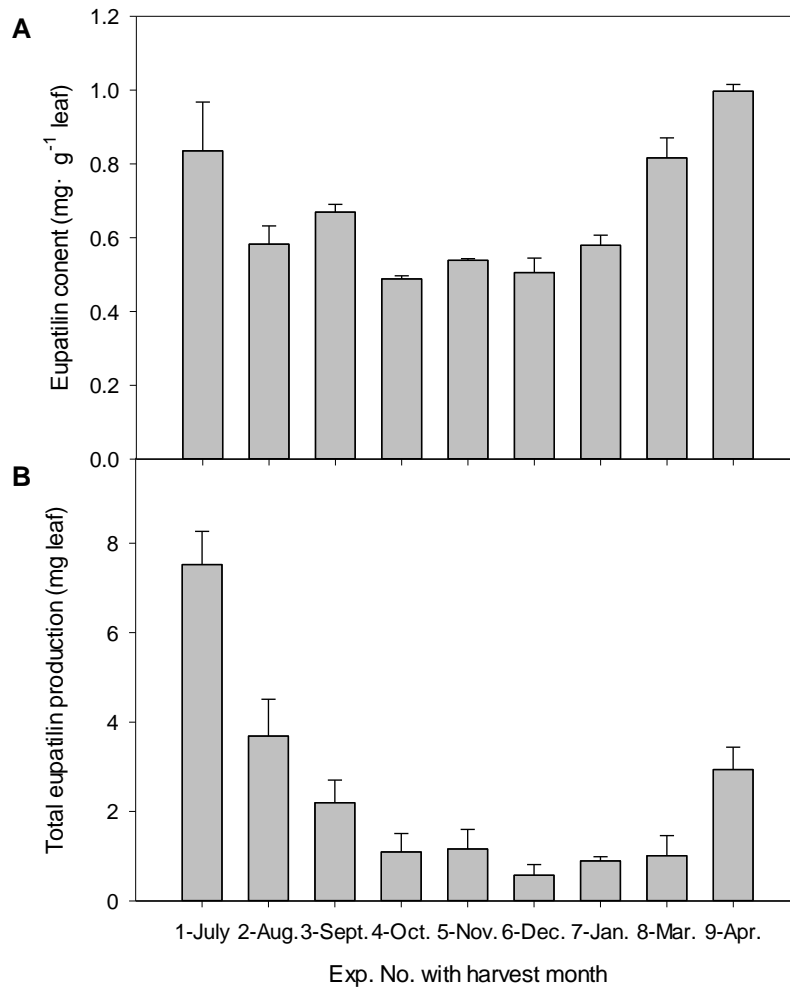


Fig. 4. Eupatilin content and total eupatilin production (eupatilin content \times leaf dry weight) in *A. princeps* cultivated during nine different seasonal cultivation periods. Vertical bars represent standard errors of the means ($n = 5$).

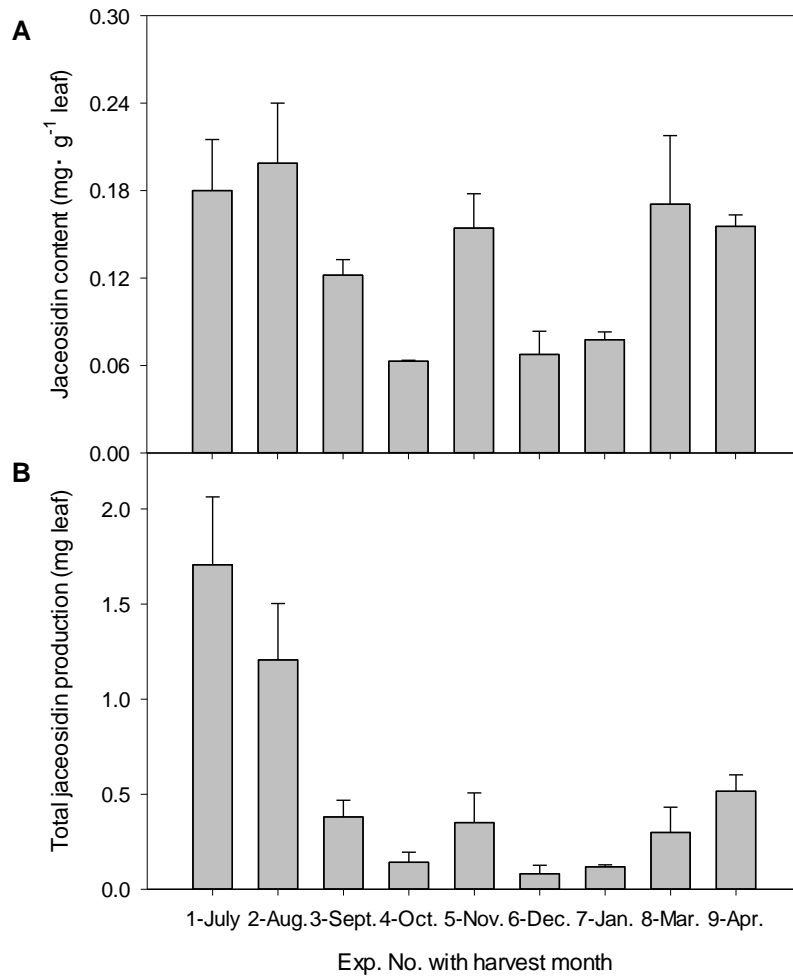


Fig. 5. Jaceosidin content and total jaceosidin production (jaceosidin content × leaf dry weight) in *A. princeps* cultivated during nine different seasonal cultivation periods. Vertical bars represent standard errors of the means (n = 5).

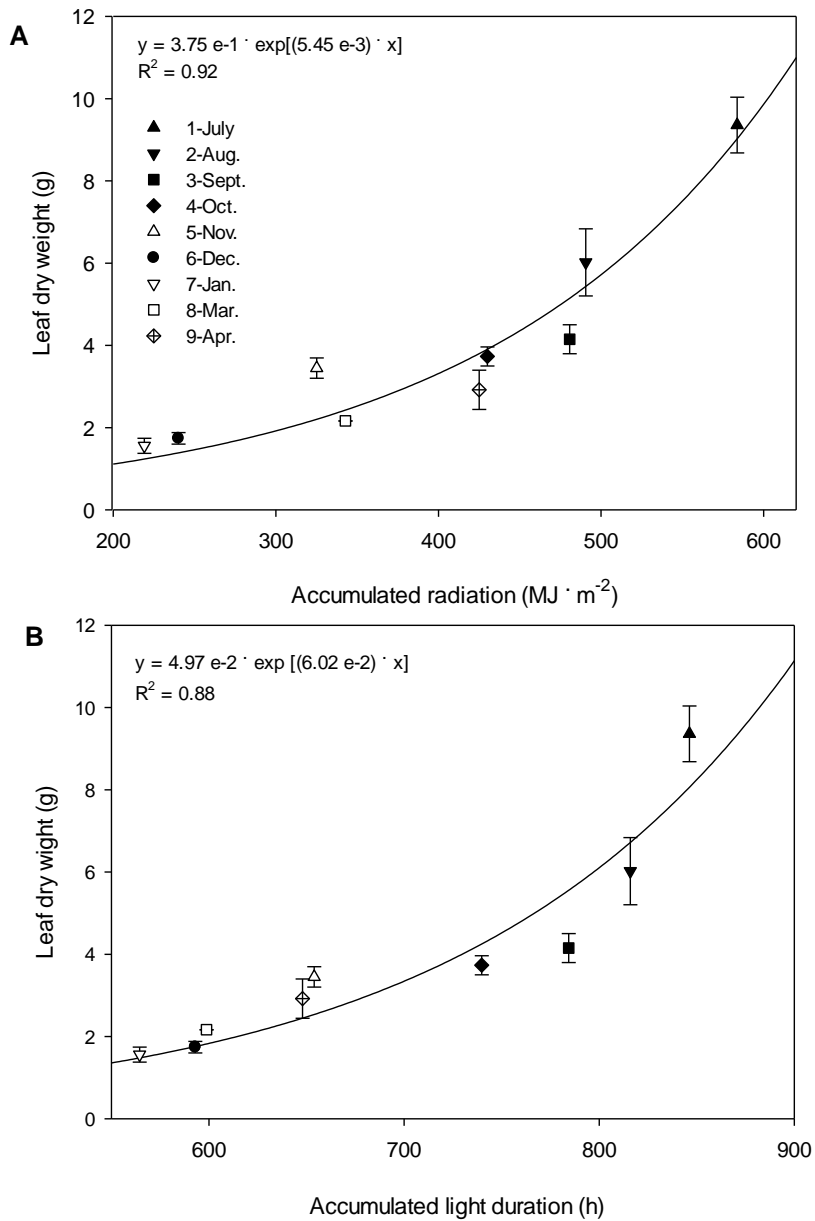


Fig. 6. Relationships of leaf dry weights of *A. princeps* with accumulated radiation (A) and light duration (B). Vertical bars represent standard errors of the means (n = 5).

The decline in plant dry matter production in response to light intensity and duration indicates the plant capacity to store photosynthesis for development is limited (Oluwasemire and Odugbenro, 2014). When irradiance is a limiting factor, in addition, the plant reduces the dark respiratory rate and compensation irradiance (Boardman, 1977). Low biomass production is resulted by low photosynthetic activity with low growth rate as the limited electron transport chain and Calvin cycle enzymes reduce the electron transport rate and the rate assessment of light response of photosystem II, lowering the photosynthetic capability (Behrenfeld et al., 2004).

The eupatilin contents shown in Exps. 1, 3, 8, and 9 were strongly related to the dry weights with accumulated radiation and light duration (Figs. 3A, 3B, 4B). Considering both biomass and eupatilin content, the total eupatilin production showed a relationship to both accumulated radiation and duration with R^2 values of 0.91 and 0.75, respectively (Figs. 7A, 7B). The eupatilin content was not constant across seasonal light conditions (Fig. 4A), and higher plant biomass accumulation resulted in higher eupatilin production (Fig. 4B), in agreement with the trend of seasonal light variation (Figs. 3A, 3B).

Unlike eupatilin, jaceosidin content had no significant relationship with seasonal light conditions (Figs. 3A, 3B, 5A). However, with the light quantity and plant growth relationship, higher total jaceosidin production

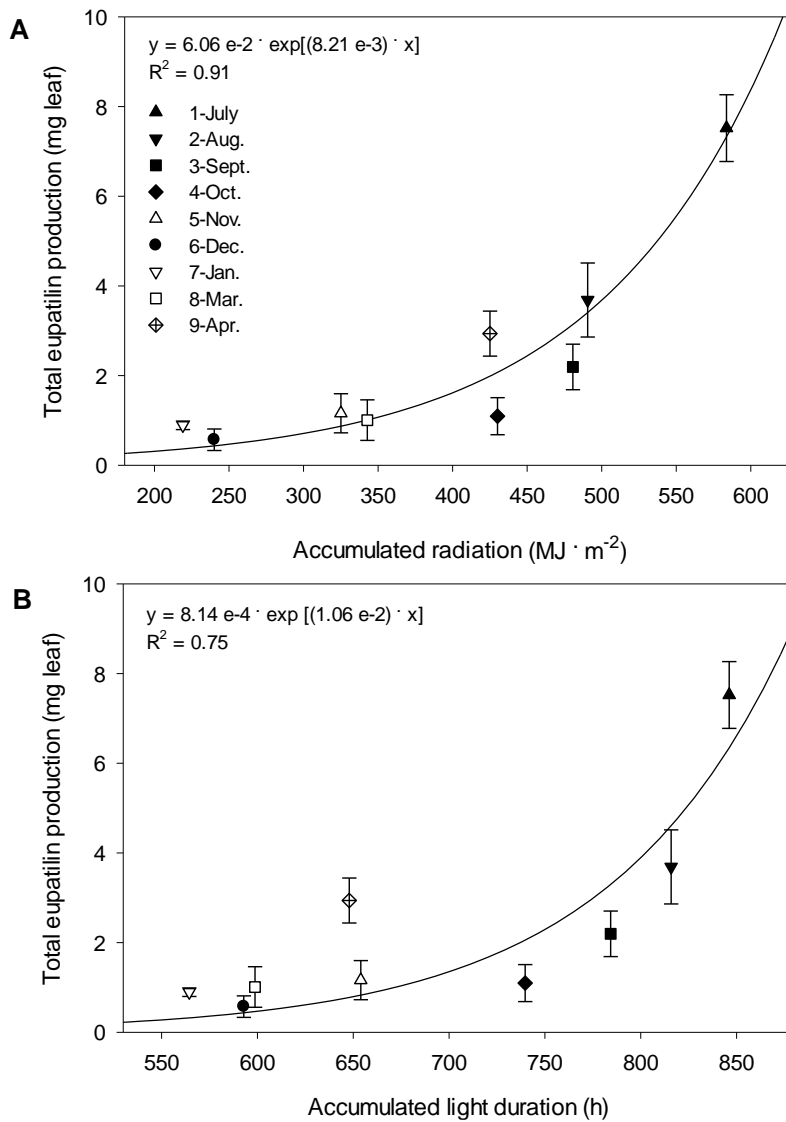


Fig. 7. Relationships of total eupatilin production in *A. princeps* with accumulated radiation (A) and light duration (B). Vertical bars represent standard errors of the means ($n = 5$).

during the cultivation period was resulted along with the accumulated radiation and duration (Figs. 3A, 3B, 5B). This relationship is illustrated in Figs. 8A and 8B with R^2 values of 0.83 and 0.82, respectively.

As the total flavonoid production was highly dependent on the plant dry matter production, environment control for optimum plant growth conditions is important. From the nine different cultivation periods, light conditions of Exp. 1 (Table 1, Figs. 3A, 3B) with at least accumulated radiation of $583.7 \text{ MJ} \cdot \text{m}^{-2}$ with accumulated light duration of 846.2 h are determined for the optimum light condition control.

Plant growth and flavonoid production under artificial light treatments

Plant growth and eupatilin contents were highly affected by the light intensity and duration. The winter season in Korea, Exps. 6 and 7, provides relatively low irradiance and short light duration. The measured accumulated radiation was 41.1% lower in Exp. 6 than in Exp. 1 (Fig. 3B, $P < 0.05$), and the accumulated light duration was 70% lower in Exp. 6 than in Exp. 1 (Fig. 3A, $P < 0.05$). Similarly, light intensity and duration were 37.5 and 66.7% lower in Exp. 7 than in Exp. 1, respectively ($P < 0.05$). Therefore, the smallest amounts of total eupatilin and jaceosidin were produced (Figs. 4B, 5B) in Exps. 6 and 7 due to lower photosynthetic activity and plant growth (Table 3).

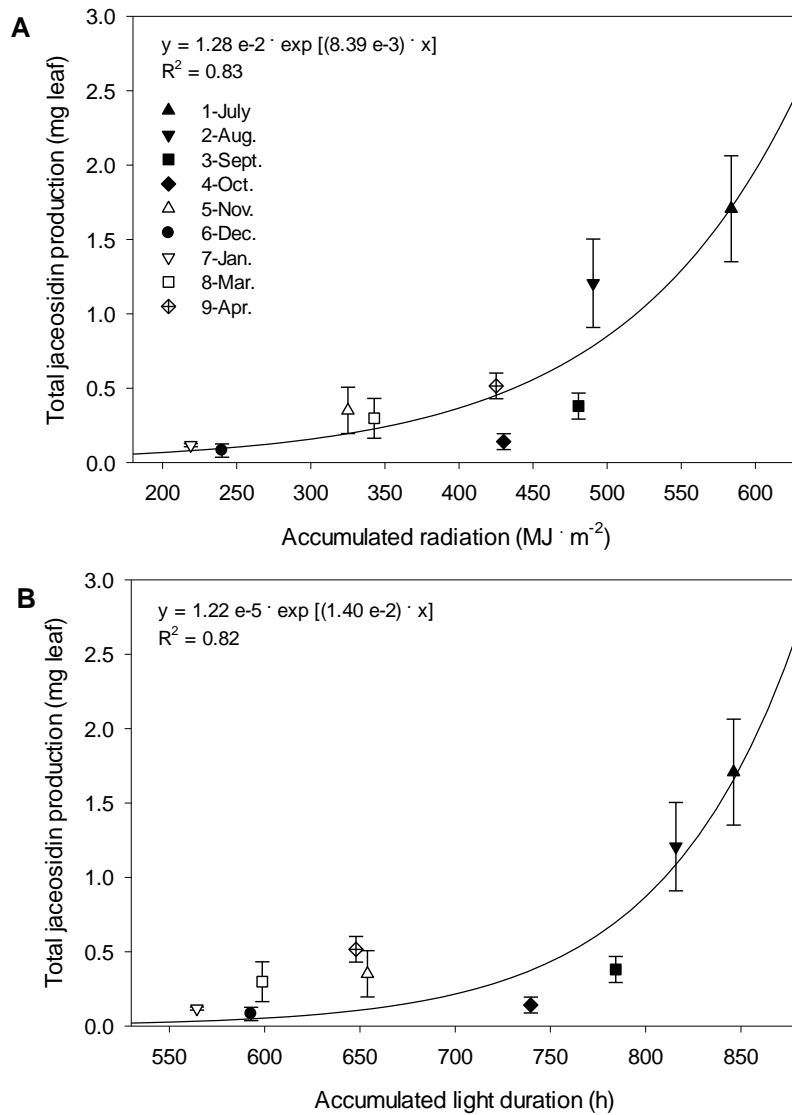


Fig. 8. Relationships of total jaceosidin production in *A. princeps* with accumulated radiation (A) and light duration (B). Vertical bars represent standard errors of the means ($n = 5$).

The plants cultivated under supplemental light treatment had greater growth than those under natural solar radiation, low radiation, and low radiation with night interruption treatments (Table 3). Supplemental light treatment during the cultivation period in Exp. 6 was an additional $28.4 \text{ MJ} \cdot \text{m}^{-2}$ and 161.1 h of light exposure, while that in Exp. 7 was an additional $27.9 \text{ MJ} \cdot \text{m}^{-2}$ and 176.6 h of light (Table 2). Both light intensity and duration increased the plant growth and eupatilin content in Exps. 6 and 7 (Table 3, Fig. 9A). Equivalent to *A. princeps*, the light quantity and *A. annua* plant biomass were positively related: an increase in irradiance increased biomass accumulations (Wang et al., 2008). Additional light intensity with duration significantly increased the plant growth and eupatilin content, resulting in a higher total eupatilin production (Fig. 9B).

Night interruption treatment in Exps. 6 and 7 also significantly increased plant growth and eupatilin content compared to the plants cultivated under natural solar radiation, low light, and low light with night interruption treatment (Table 2, Fig. 9A). Night interruption treatment in Exps. 6 and 7 increased the accumulated radiation by $1.1 \text{ MJ} \cdot \text{m}^{-2}$ compared to natural light and 232 and 228 h of additional light exposure, respectively. In Exps. 6 and 7, night interruption was employed with light intensity of $10 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for 4 h daily; thus, the night interruption treatment did not significantly influence accumulated radiation and biomass accumulation. Light duration could affect the plant growth by

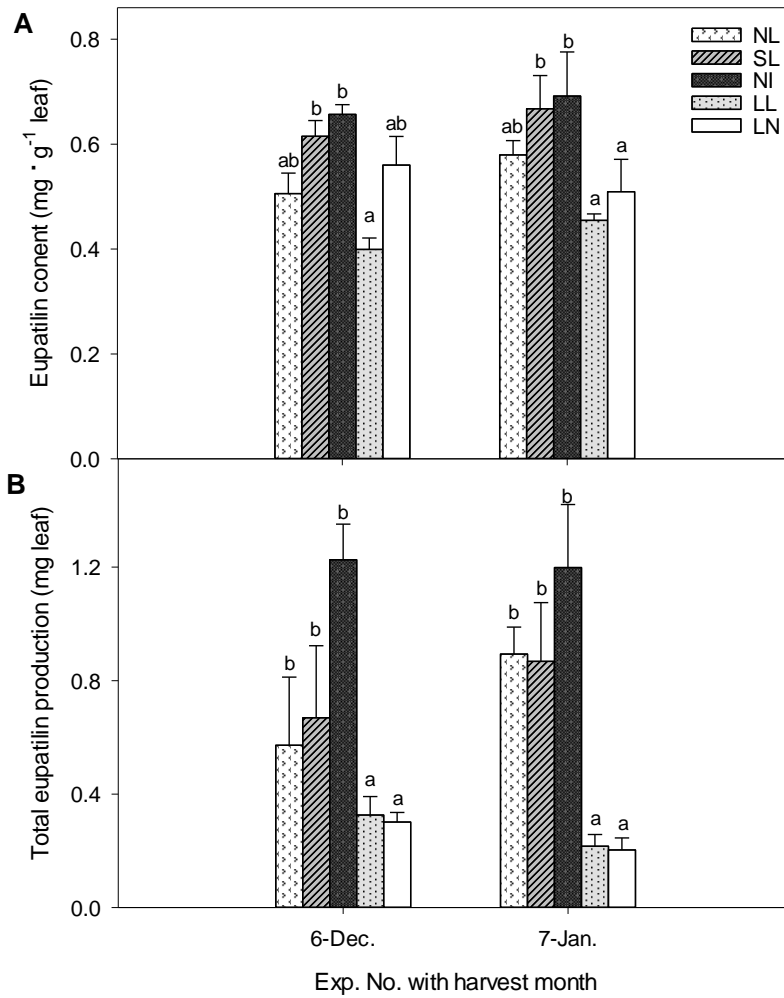


Fig. 9. Eupatilin content (A) and total eupatilin production (B) in *A. princeps* of Exps. 6 and 7. Vertical bars represent standard errors of the means (n = 5). Letters represent significant differences by Tukey's test at $P < 0.05$.

providing a signal to the plants to maintain their vegetative stage and limited the transition to reproductive stage.

The increase in secondary metabolites under long photoperiodic conditions can be a result of an increase in incident light energies (Jaakola and Hohtola, 2010). However, decreases in flavonoid content with a seasonal change of light duration have also been reported in several *Artemisia* species studies: *A. annua*, *A. montana*, and *A. capillaris*; the content increased until flowering and decreased after the reproductive stage (Choi et al., 2008; Ferreira et al., 1995; Kim et al., 2013). Various crops are also sensitive to light duration for vegetative growth as specific photoperiods produce high biomass through continued vegetative growth for sorghum (Meki et al., 2017) and June-bearing strawberry plants (Konsin et al., 2001). Therefore, light duration enhanced the plant growth and biomass accumulation of *A. princeps* (Fig. 9B), similarly to the supplemental light treatment. However, artificial lighting affects the production cost (Lee et al., 2017), so from the viewpoint of industrial production, the use of night interruption treatment allows similar plant growth and eupatilin content while requiring lower energy input than supplemental light treatment.

Although eupatilin content was significantly related to both light intensity and duration, jaceosidin content was not statistically significant in relation to light quantity (Fig. 10A). However, with the plant growth and

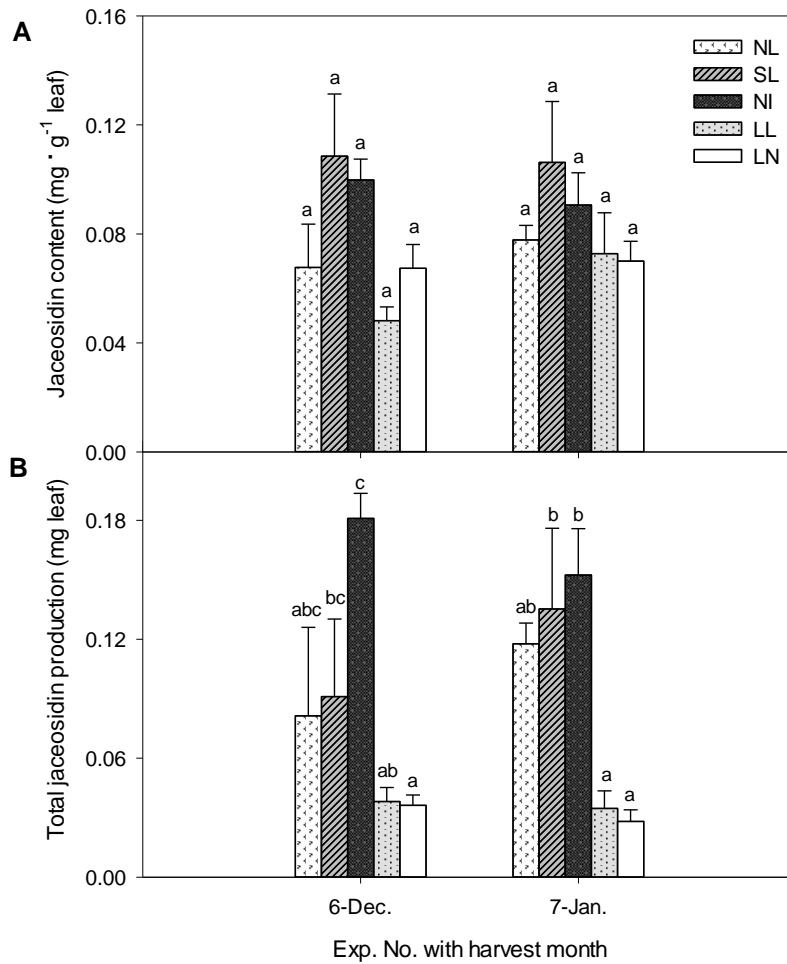


Fig. 10. Jaceosidin content (A) and total jaceosidin production (B) in *A. princeps* of Exps. 6 and 7. Vertical bars represent standard errors of the means ($n = 5$). Letters represent significant differences by Tukey's test at $P < 0.05$.

biomass accumulation in the leaves, total jaceosidin production increased with night interruption light treatment (Fig. 10B).

CONCLUSIONS

Seasonal changes in environmental conditions, specifically light intensity and duration, affected the growth and flavonoid production of *A. princeps* grown in greenhouses. As the accumulated radiation and duration increased, photosynthesis increased, resulting in increased plant height, number of nodes, stem diameter, SPAD value, total biomass, and flavonoid production. Light intensity enhanced the plant growth and light duration maintained the vegetative growth of the plant. Medicinal plants cultivated in fields have significant challenges with consistency in safety, efficacy, quality, and quantity of production (Mosaleeyanon et al., 2005). The present study showed that the biomass and production of target medicinal bioactive compounds can be consistently and economically produced when harvested in greenhouses with adequate night interruption light treatments when natural light duration is less than 13 h.

LITERATURE CITED

- Ahn JB, Hur JN, Jung HG, Park JH** (2012) Study on the growth environment of 'Gangwha-mugwort' through the climatological characteristic analysis of Gangwha region. *Kor. J. Agric. For. Meteorol.* 14:71-78.
- Bang MH, Cho JG, Song MC, Lee DY, Han MW, Chung HG, Lee KT, Choi MS, Baek NI** (2008) Development of biologically active compounds from edible plant sources. XXII. Triterpenoids from the aerial parts of Sajabalssuk (*Artemisia princeps* Pampanini). *Appl. Biol. Chem.* 51:223-227.
- Bang MH, Chung HG, Song MC, Yoo JS, Chung SA, Lee DY, Kim SY, Jeong TS, Lee KT, Choi MS, Baek NI** (2006) Isolation of sterols from the aerial parts of Sajabalssuk (*Artemisia herba*). *J. Kor. Soc. Appl. Biol. Chem.* 49:140-144.
- Behrenfeld MJ, Prasil O, Babin M, Bruyant F** (2004) In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis. *J. Phycol.* 40:4-25.
- Boardman NK** (1977) Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Physiol.* 28:355-377.

- Cho YH, Chiang MH** (2001) Essential oil composition and antibacterial activity of *Artemisia capillaris*, *Artemisia argyi*, and *Artemisia princeps*. Kor. J. Intl. Agric. 13:313-320.
- Choi SR, You DH, Kim JY, Park CB, Ryu J, Kim DH, Eun JS** (2008) Antioxidant and antimicrobial activities of *Artemisia capillaris* Thunberg. Kor. J. Med. Crop Sci. 16:112-117.
- Davik J, Bakken AK, Holt K, Blomhoff R** (2006) Effects of genotype and environment on total anti-oxidant capacity and the content of sugars and acids in strawberries (*Fragaria × ananassa* Duch.). J. Hortic. Sci. Biotechnol. 81:1057-1063.
- Dorais M, Papadopoulos AP, Luo X, Leonhart S, Gosselin A, Pedneault K, Angers P, Gaudreau L** (2001) Soilless greenhouse production of medicinal plants in North Eastern Canada. Acta Hortic. 554:297-304.
- Ferreira JF, Simon JE, Janick J** (1995) Developmental studies of *Artemisia annua*: Flowering and artemisinin production under greenhouse and field conditions. Planta Med. 61:167-170.
- Fonseca JM, Rushing JW, Rajapakse NC, Thomas RL, Riley MB** (2006) Potential implications of medicinal plant production in controlled environments: The case of feverfew (*Tanacetum parthenium*). HortScience 41:531-535.

- Gonzalez-Coloma A, Bailen M, Diaz CE, Fraga BM, Martínez-Díaz R, Zuñiga GE, Contreras RA, Cabrera R, Burillo J** (2012) Major components of Spanish cultivated *Artemisia absinthium* populations: Antifeedant, antiparasitic, and antioxidant effects. *Ind. Crop Prod.* 37:401-407.
- Höft M, Verpoorte R, Beck E** (1996) Growth and alkaloid contents in leaves of *Tabernaemontana pachysiphon* Stapf (*Apocynaceae*) as influenced by light intensity, water, and nutrient supply. *Oecologia* 107:160-169.
- Jaakola L, Hohtola A** (2010) Effect of latitude on flavonoid biosynthesis in plants. *Plant Cell Environ.* 33:1239-1247.
- Jelodar NB, Bhatt A, Mohamed K, Keng CL** (2014) New cultivation approaches of *Artemisia annua* L. for a sustainable production of the antimalarial drug artemisinin. *J. Med. Plant Res.* 8:441-447.
- Kim DH, Na HK, Oh TY, Kim WB, Surh YJ** (2004) Eupatilin, a pharmacologically active flavone derived from *Artemisia* plants, induces cell cycle arrest in ras-transformed human mammary epithelial cells. *Biochem. Pharmacol.* 68:1081-1087.
- Kim YJ, Lee JH, Kim SJ** (2013) Cultivation characteristics and flavonoid contents of wormwood (*Artemisia montana* Pamp.). *J. Agric. Chem. Environ.* 2:117-122.

- Konsin M, Voipio I, Palonen P** (2001) Influence of photoperiod and duration of short-day treatment on vegetative growth and flowering of strawberry (*Fragaria × ananassa* Duch.). J. Hortic. Sci. Biotechnol. 76:77-82.
- Lee JW, Kang WH, Park KS, Son JE** (2017) Spectral dependence of electrical energy-based photosynthetic efficiency at single leaf and canopy levels in green- and red-leaf lettuces. Hortic. Environ. Biotechnol. 58:111-118.
- Lee SH, Bae EA, Park EK, Shin YW, Baek NI, Han EJ, Chung HG, Kim DH** (2007) Inhibitory effect of eupatilin and jaceosidin isolated from *Artemisia princeps* in IgE-induced hypersensitivity. Intl. Immunopharmacol. 7:1678-1684.
- Ma Z, Li S, Zhang M, Jiang S, Xiao Y** (2010) Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of *Anoectochilus* plants. HortScience 45:863-867.
- Meki MN, Ogoshi RM, Kiniry JR, Crow SE, Youkhana AH, Nakahata MH, Littlejohn K** (2017) Performance evaluation of biomass sorghum in Hawaii and Texas. Ind. Crop Prod. 103:257-266.
- Mierziak J, Kostyn K, Kulma A** (2014) Flavonoids as important molecules of plant interactions with the environment. Molecules 19:16240-16265.

- Mosaleeyanon K, Zobayed SMA, Afreen F, Kozai T** (2005) Relationships between net photosynthetic rates and secondary metabolite concentrations in St. John's wort. *Plant Sci.* 169:523-531.
- Oh TY, Ahn BO, Ko JI, Ryu BK, Son MW, Kim SH, Kim WB, Lee EB** (1997) Studies on protective effect of DA-9601, an *Artemisia* herba extract, against ethanol-induced gastric mucosal damage and its mechanism. *J. Appl. Pharmacol.* 5:202-210.
- Oluwasemire KO, Odugbenro GO** (2014) Solar radiation interception, dry matter production, and yield among different plant densities of *Arachis* spp. in Ibadan, Nigeria. *Agric. Sci.* 5:864.
- Ryu SN** (2008) Environmental variation of available component in Mugwort (*Artemisia princeps* Pamp.). *J. Kor. Soc. Intl. Agric.* 20:40-46.
- Ryu SN, Han SS, Yang JJ, Jeong HG, Kang SS** (2005) Variation of eupatilin and jaceosidin content of mugwort. *Kor. J. Crop Sci.* 50: 204-207.
- Treutter D** (2005) Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biol.* 7:581-591.
- Wang ML, Jian YS, Wei JQ, Wei X, Qi XX, Jiang SY, Wang ZM** (2008) Effects of irradiance on growth, photosynthetic characteristics, and artemisinin content of *Artemisia annua* L. *Photosynthetica* 46:17-20.

Zobayed SMA, Afreen F, Kozai T (2005) Necessity and production of medicinal plants under controlled environments. *Environ. Contr. Biol.* 43:243-252.

Zobayed SSPK, Saxena PK (2004) Production of St. John's wort plants under controlled environment for maximizing biomass and secondary metabolites. *In vitro Cell Dev. Biol. Plant* 40:108-114.

ABSTRACT IN KOREAN

Artemisia princeps(강화약쑥)은 위궤양 및 위경련 치료의 특성을 가지고 있는 플라보노이드 유파틸린과 자세오시딘을 함유하고 있다. 일반적으로 노지에서 연중 1회 재배되며 환경 변화에 따라 플라보노이드 함량이 불안정하다. 본 연구의 목적은 강화약쑥의 연중 안정적인 온실 재배를 위해 계절적인 광 조건 변화와 인공 조명 처리에 대한 식물의 성장과 플라보노이드 함량 변화를 분석하는 것이다. 연중 총 9회의 재배 작기로 온실의 계절적인 광 조건 하에서 재배하였다. 광 조건의 변화가 크게 나타나는 겨울철 2회의 작기 중에는 인공 조명 처리 추가로 보광, 야파, 저광 및 야파 조건에서 재배하였다. 수확 후, 식물의 성장을 측정하고, 유파틸린과 자세오시딘의 함량을 분석하였다. 누적 일사량과 누적 일장이 높을수록 식물의 성장이 좋았으며, 성장과 플라보노이드 함량은 누적 일사량과 누적 일장과 유의한 관계를 보였다. 보광 및 야파 처리로 인해 성장과 플라보노이드 함량이 현저히 높게 나타났다. 일장이 13시간 이하인 겨울철에 총 성장과 플라보노이드 함량 향상을 위해 온실 재배 시 야파 처리로 생산 증대와 연중 안정적인 생산이 가능하다고 판단된다.

추가 주요어: 강화약속, 광량, 온실 재배, 연중 생산, 유파틸린, 일장,
자세오시딘

학번: 2015-23014