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# **Application of Extended Lighting Cycles to Promote Vegetative Growth of Vegetables Grown in Closed Plant Production Systems**

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## **ABSTRACT**

In the present study, extended lighting cycle (ELC), a novel lighting pattern was proposed for the production of vegetables in closed plant production systems where artificial lights were adapted as a sole lighting source. The effects of ELC including continuous lighting (CL) on growth of head lettuces, leaf lettuces, Chinese cabbages, pak-chois, hot peppers and tomatoes were examined in the first chapter of this dissertation. When they were grown under various lighting cycle conditions; CL, 16, 24, and 32-h photoperiods combined with an 8-h dark

period, and 20, 30 and 40-h photoperiods combined with a 10-h dark period, plant growth and light use efficiency (LUE) increased as the ratio of photoperiod to lighting cycle increased at the earlier growth stage. After that, the greater ratio of photoperiod to lighting cycle, the earlier growth inhibition occurred except head lettuces. In tomatoes growth retardation and chlorosis occurred within 14 days after sowing (DAS) in treatment CL. Those symptoms were found in other ELC treatments and they occurred earlier and more seriously as the ratio of photoperiod to lighting cycle increased. Occurrence time and the severity of those symptoms were varied by plant species, too. Tomatoes were most susceptible to ELC-related symptoms among the six tested vegetable, followed by hot peppers, pak-chois, Chinese cabbages, leaf lettuces, and head lettuces. In head lettuces those symptoms were not found until the end of experiment (28 DAS).

In the second chapter, the effect of insertion of dark period in the middle of continuous lighting to avoid growth inhibition caused by continuous lighting in tomatoes was examined. Tomato seedlings were grown under various lighting scenarios; four different insertion timings of dark period (changed from CL to 16/8-h lighting cycle at 6, 8, 10, and 12 DAS) and six different lengths of dark period inserted (8, 16, 24, 32, 40, and 48-h) after 10 days of CL. Growth inhibition caused by CL was avoided by insertion of dark period in the middle of

CL. The growth of tomatoes was greatest when the lighting cycle condition was changed from CL to 16/8-h at 6 DAS, and the effectiveness of avoidance decreased as the insertion time of dark period were delayed. The effectiveness of the avoidance did not change, however, as the duration of dark periods inserted was prolonged from 8 up to 48 h. The results of this study imply that ELC or CL with insertion of dark period can be applied for rapid and efficient production of leafy and fruit vegetables and/or their transplants production in closed plant production systems using artificial lights.

Keyword: extended lighting cycle, continuous lighting, dark period, light use efficiency, growth inhibition, closed plant production system

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## INTRODUCTION

Photoperiod (i.e., period of daylight in every 24 hours) is one of the primary factors that affect vegetative growth of plants. There have been several reports on its effects on biomass production (Degli et al., 1990; Drozdova et al., 2004), leaf emergence (Koontz and Prince, 1986), leaf size (Junttila et al., 1997), and changes in secondary compounds (Taylor et al., 1994). When the photoperiods increased, plant biomass production rate increased in many plant species (Koontz and Prince, 1986; Masuda and Murage, 1998; Ohler and Mitchell, 1996). Langton et al. (2003) reported that the increase in biomass production resulted from actions related to increases in leaf area and chlorophyll content.

The increased photoperiods, however, do not always promote plant growth. An extension of the photoperiod from 20 to 24 h d<sup>-1</sup> did not promote the total dry weight, number of leaves, and plant height in foliage plants (Mortensen and Grimstad, 1990). Demers et al. (1998) reported that biomass production and plant height had become significantly greater in 14-h photoperiod treatment than those in 24-h photoperiod treatment in tomato. A typical chlorosis in the tomato leaves appeared at photoperiods longer than 18 h when incandescent lamps are used as the sole source (Withrow and Withrow, 1949). Especially, under

continuous lighting condition, some plants including eggplant (Murage et al., 1997), potato (Stutte et al., 1996), tomato (Bradley and Janes, 1985), and cowpea (Ohler and Mitchell, 1996) had responded with limited biomass production and with chlorosis and necrosis.

Chlorosis and/or necrosis caused by continuous lighting (CL) attributed to the damaging action of oxygen in an excited state such as the superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl ( $\cdot OH$ ) radicals have been well reported (Foyer et al., 1994; Powles, 1984; Scandalios, 1993). Under CL condition, photosynthetic efficiency was reduced as interception of the incoming light continues unabated (Dorais et al., 1996). It is, therefore, possible that a direct leakage of electrons to molecular oxygen occurs, enhancing the generation of toxic oxygen species. This can damage the ultrastructure and function of chloroplasts, and photosynthetic pigments leading to leaf chlorosis (Foyer et al., 1994; Krause, 1988; Scandalios, 1993).

Growth inhibition caused by CL can be suppressed by applying dark periods. Light stress-induced growth inhibition is generally not observed in the dark or in natural environments (Foyer et al., 1994). Murage et al. (1997) reported that 9 h per day of dark period were necessary in order to prevent leaf injury characterized by chlorosis and necrosis in eggplant.

In closed plant production systems (CPPSs), environmental conditions can

controlled as desired regardless of outside weather conditions, because its outer structure consists of opaque and thermally insulated materials, and energy and mass exchanges between inside and outside the system are very limited (Kozai et al., 1999). Extended lighting cycle (ELC), i.e., lighting cycle longer than 24 h can be applied in a CPPS with artificial lighting. The growth responses to ELC have usually less pronounced, and their effects on the growth and development of plants and resource use efficiencies are needed to be investigated.

Objectives of this study were to evaluate the potential use of ELC for production of vegetable transplants (Chapter 1), and to avoid growth inhibition of tomatoes caused by continuous lighting by insertion of dark period in the middle of CL (Chapter 2).

# LITERATURE REVIEW

## Crop productivity as affected by lighting cycle

Global demand and consumption of agricultural crops for food, feed, and fuel is increasing at a rapid pace (Edgerton, 2009). Horticulturists have long sought to maximize productivity of crop plants since the beginning of agriculture. Crop improvement through plant breeding will be the major contributor to increased crop productivity. The optimization of environmental conditions, light quality (Ohashi-Kaneko et al., 2007; Li and Kubota. 2009), photoperiod (Vaillant et al., 2005; Kitaya et al., 1998), the root-zone environment (Datt et al., 2003), CO<sub>2</sub> concentration (Lawlor and Mitchell, 1991; Li et al., 2007), and temperature (Morison and Lawlor, 1999; Thompson and Langhans, 1998) are also contributor to increased crop productivity.

Light is regarded as a primary factor regulating plant growth and development. Kitaya et al. (1998) showed that growth increased and quality improved as PPF increased in lettuce. Extending photoperiod has resulted in substantial increases in fresh weight for radishes (Inada and Yabumoto, 1989) and various lettuce cultivars (Koontz and Prince, 1986). On the other hand, the increased photoperiods have not always promoted plant growth in tomato

(Demers et al., 1998) and some foliage plants (Mortensen and Grimstad, 1990). These results indicated that appropriate photoperiod was necessary to promote crop productivity depending on plant species.

### **Closed plant production systems**

The closed plant production system (CPPS) was designed to produce high-quality vegetables all year round by artificially controlling the culture condition, such as light quality, photoperiod, temperature, CO<sub>2</sub> concentration, and culture solution (Kozai et al., 1999). By controlling the internal environment, vegetables can be produced in CPPSs a few times faster than typical outdoor cultivation. In addition, as multiple cultivation shelves are used, the mass production of vegetables in a small space is facilitated. Hundreds of CPPSs have currently been operated in Korea and Japan producing lettuces, spinaches, strawberries, transplants of fruit vegetables and so on.

In a CPPS, optimal environmental control techniques to obtain higher yields and better quality of crops are essential from the viewpoint of cost-performance (Morimoto et al., 1995). Despite the previous studies conducted (Morimoto and Hashimoto, 2000; Okayama et al., 2008), it is thought to be difficult to determine the control parameters for optimizing physiological processes of plants characterized by complexity and uncertainty.

## **Continuous lighting-induced negative effects**

Some plant species grown under CL condition showed various negative symptoms. Notable examples of CL-induced negative effects are chlorosis and necrosis in tomato (Murage et al., 1996; Dorais et al., 1996). The CL also lowers photosynthetic parameters, including photosynthetic capacity at saturating light in cucumber (Pettersen et al., 2010a), maximum rate of Rubisco carboxylation in onion (Van Gestel et al., 2005), quantum yield in cucumber (Pettersen et al., 2010b), and maximum rate of electron transport in peanut (Rowell et al., 1999). The CL might increase photo-oxidative pressure and tobacco plants grown under CL condition showed higher reactive oxygen species (ROS)-detoxifying enzyme activity than those grown under diurnal photoperiods (Peter et al., 2010). In *Arabidopsis*, a knockout mutant with reduced content of 2-Cys peroxiredoxin, an antioxidant enzyme, showed lower CO<sub>2</sub> fixation rate under CL condition (Pulido et al., 2010). Because of those reasons, for cultivating CL-sensitive plant species in CPPSs proper combination of photoperiods and dark periods are needed to achieve optimal growth and development.

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# **CHAPTER 1**

## **Growth of Vegetables under Extended Lighting Cycle Conditions**

### **INTRODUCTION**

Extending photoperiods with artificial lighting increases growth and yield of many horticultural species such as lettuce (Kitaya et al., 1998; Gaudreau et al., 1994), Chinese cabbage (Moe and Guttormsen, 1985), pepper (Dorais et al., 1996; Demer and Gosselin, 2000), and cucumber (Pramanik et al., 2000), and this technique has been used in greenhouse production. An extension of photoperiod from 20 h d<sup>-1</sup> to continuous lighting (CL), however, did not promote growth and development in some foliage plants (Mortensen and Grimstad, 1990). Under extended photoperiods including CL, sensitive species like tomatoes tend to develop important physiological disorder (Demer et al., 1998; Jensen and Veiershow, 1998). In this sense, to determine the proper length of dark period

was necessary to achieve optimal productivity and to prevent growth inhibitors caused by extended photoperiod.

The commercial possibility of producing various vegetable species under artificial lighting in closed plant production systems (CPPSs) has been discussed by Kozai (1998) and others. The vegetable production in CPPSs has many advantages such as rapid growth, high quality and safety, uniformity of products, and so on. In those systems, lighting conditions such as photosynthetic photon flux (PPF), spatial distribution and direction, photoperiod and dark period, and spectral composition can be controlled regardless of outside weathers. Thus, vegetative, reproductive, and morphological development of vegetables produced in CPPSs can be manipulated.

Plant responses to the lighting cycle that are longer than 24 h, henceforth extended lighting cycles (ELCs), has seldom reported. In this study, we hypothesized that the ELCs affect growth and development of vegetable and investigated the plant responses to the ELCs using six vegetables; head lettuces, leaf lettuces, Chinese cabbages, pak-chois, hot peppers and tomaotes.

## MATERIALS AND METHODS

### Plant materials and culture conditions

Head lettuces “Ulake” (Mikado Kyowa Seed Co., Ltd., Japan), leaf lettuces “Chungchima” (Monsanto Korea Co., Ltd., Korea), Chinese cabbages “Bommat” (Nongwoobio Co., Ltd., Korea), pak-chois “Nongwoo” (Nongwoobio Co., Ltd., Korea), hot peppers “PR Chunmyung” (Nongwoobio Co., Ltd., Korea), and tomatoes “Momotaro” (Takii Seed Co., Japan) were grown for 28 days under seven lighting cycle conditions; 16, 24, and 32-h photoperiods combined with an 8-h dark period, 20, 30 and 40-h photoperiods combined with a 10-h dark period, and CL. Seeds of the six vegetables were sown on 72-cell trays filled with growing media (Sikmulsekye; Nong Woo Green-Tech Co. Ltd., Kores) and subsequently cultured in growth chambers equipped with white fluorescent lamps. PPF during photoperiod at empty shelves was  $187 \pm 13 \mu\text{mol m}^{-2} \text{s}^{-1}$  and air temperature was maintained at 28/25°C during photo-/dark periods. The plants were subirrigated for 20min once a day with the standard nutrient solution developed by National Horticultural Experiment Station, Japan. Data were analyzed using SAS 9.1 version (SAS Inst. Inc., Cary, NC, USA) for Duncan’s multiple range test (DMRT) at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

### **Plant growth of head lettuces and leaf lettuces**

Both in head lettuces and leaf lettuces, shoot dry weight, light use efficiency (LUE), and crop growth rate (CGR) were greatest in treatment CL until 21 DAS (days after sowing). All the growth parameters of head lettuces investigated were greatest in treatment CL at 28 DAS, too, while those of leaf lettuces were greatest in treatment 32/8 (Figs. 1-1, 1-2, and 1-3). Shoot fresh weight, number of leaves and leaf area also showed a similar trend (Tables 1-1 and 1-2).

As the ratio of photoperiod to lighting cycle was increased, number of leaves, leaf area, and shoot fresh weight increased until 21 DAS in both plants. At 28 DAS the same tendency was observed in head lettuces, while shoot dry weight, LUE and CGR in treatment CL became smaller than those in some of other treatments in leaf lettuce (Fig. 1-1 and Table 1-1).

There have been plenty of studies for promoted growth of lettuces under prolonged photoperiod (Gaudreau et al., 1994; Okayama et al., 2010). Tibbitts and Rao (1968) reported that the prolonged photoperiod increased the incidence of tipburn in lettuces. Tipburn is known as a physiological disorder associated with low calcium levels and causes young leaves to become brown and to have

necrosis beginning at the leaf margins (Collier and Tibbitts, 1982). It is associated with environmental conditions conducive to the rapid accumulation of dry matter (Cox et al., 1976). Tipburn is developed more rapidly under high-energy lighting conditions (Gaudreau et al., 1994).

The CL can be used for rapid production of head lettuces at least up to until 28 DAS even more energy-efficiently without any negative effects in growth and development. In leaf lettuces, the CL can be applied to promote the growth at least up to 21 DAS, while ELC (e.g. treatment 32/8) can be appropriate to be applied for long-term cultivation.

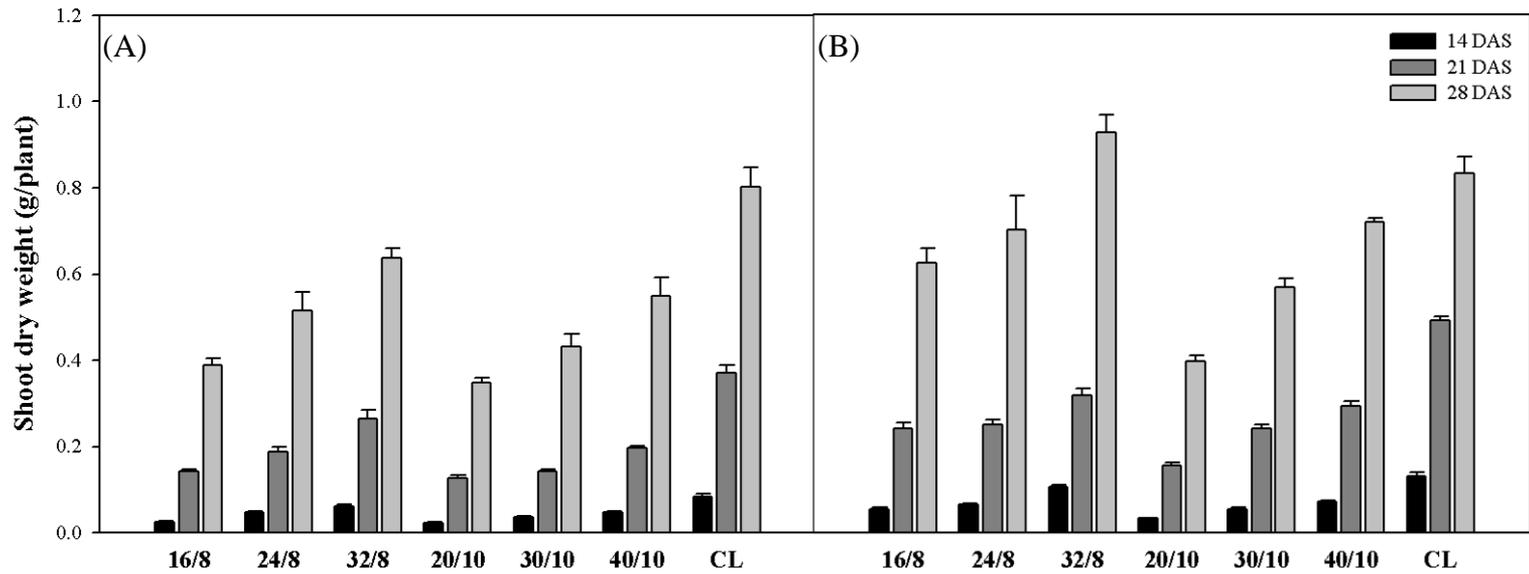


Fig. 1-1. Shoot dry weight of head lettuces (A) and leaf lettuces (B) grown for 28 days under various lighting cycle conditions.

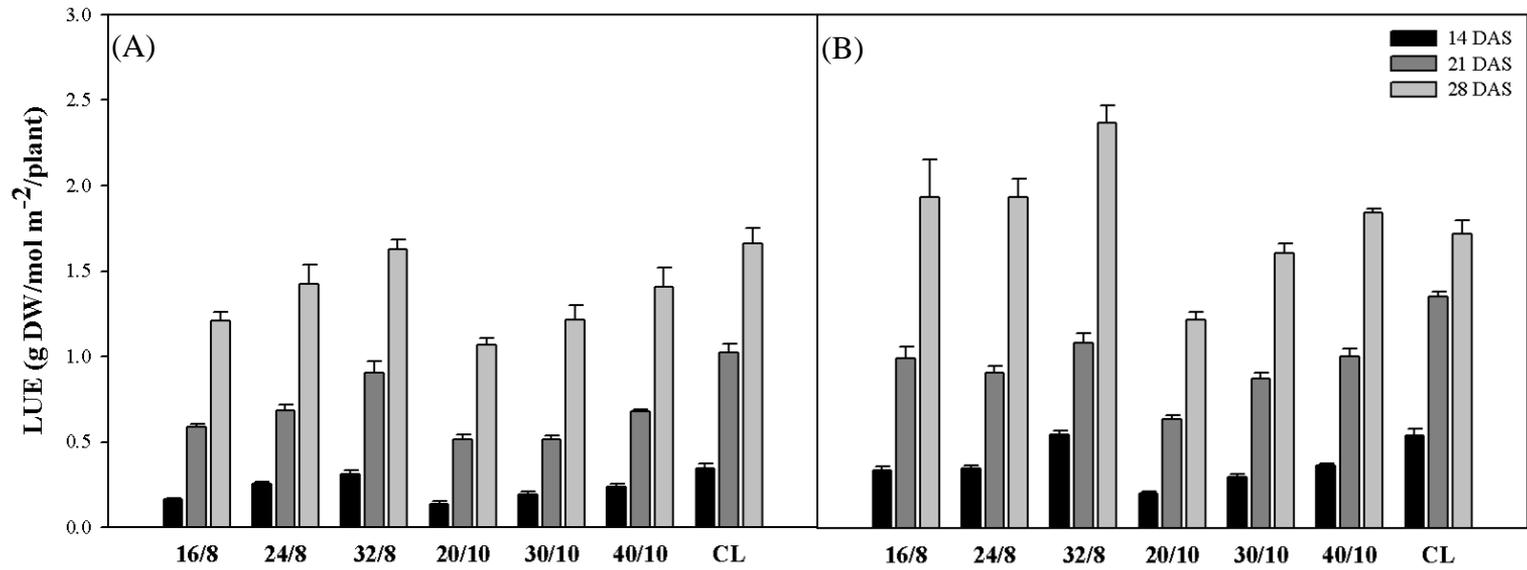


Fig. 1-2. Light use efficiency (LUE) of head lettuces (A) and leaf lettuces (B) grown for 28 days under various lighting cycle conditions.

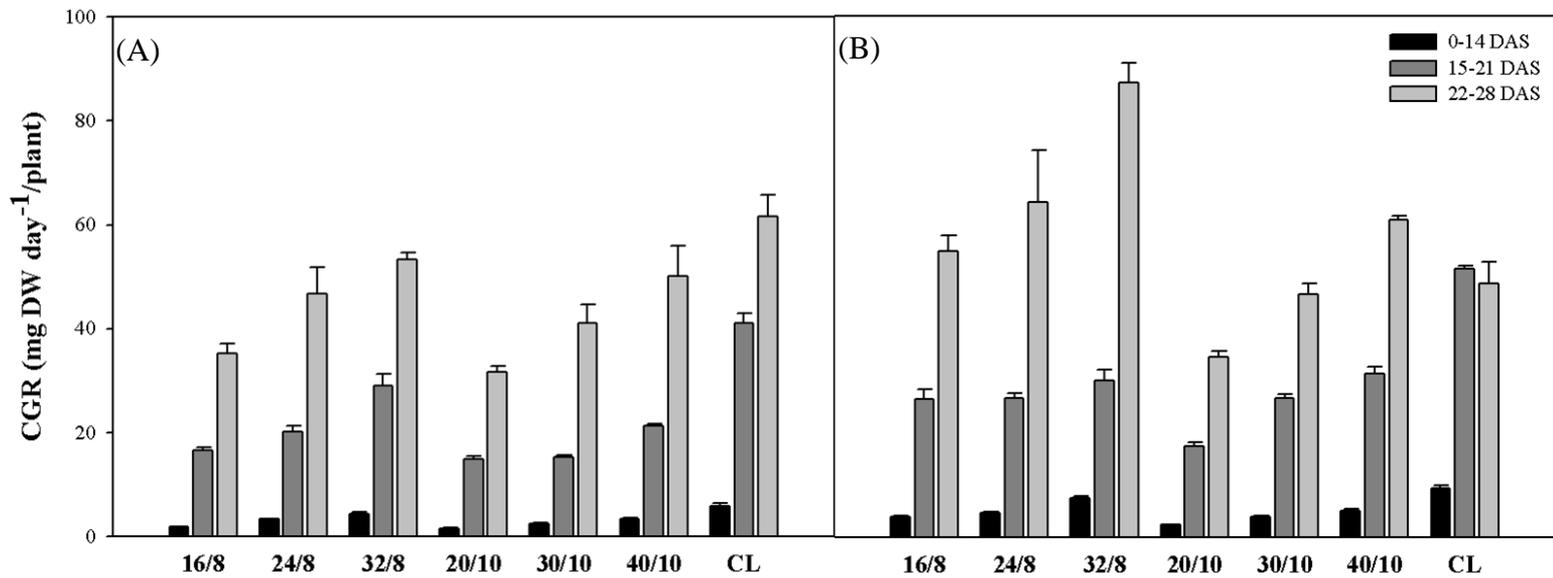


Fig. 1-3. Crop growth rate (CGR) of head lettuces (A) and leaf lettuces (B) grown for 28 days under various lighting cycle conditions.

Table 1-1. Number of leaves, leaf area, shoot fresh and dry weights, and percentage of dry matter of head lettuces grown under various lighting cycle conditions.

	No. of leaves	Leaf area (cm <sup>2</sup> )	Shoot fresh weight (g)	Shoot dry weight (g)	Percentage of dry matter (%)
14 DAS					
16/8	4.6bc <sup>Z</sup>	12.77e	0.471e	0.026de	5.6a
24/8	5.0ab	20.66c	0.823c	0.048c	5.8a
32/8	5.2a	26.62b	0.964b	0.062b	6.4a
20/10	4.0d	11.55e	0.420e	0.023e	5.5a
30/10	4.2cd	16.32d	0.612d	0.036d	5.9a
40/10	5.0ab	20.22c	0.808c	0.048c	5.9a
CL	5.4a	33.95a	1.375a	0.084a	6.1a
21 DAS					
16/8	7.8ab	68.96de	2.716d	0.142d	5.3c
24/8	6.8bc	79.32cd	3.346c	0.189c	5.7bc
32/8	7.8ab	99.00b	4.367b	0.265b	6.0b
20/10	7.0abc	53.94f	2.131e	0.127d	5.9bc
30/10	6.6c	59.74ef	2.354de	0.143d	6.1b
40/10	7.8ab	83.01c	3.540c	0.197c	5.6bc
CL	8.0a	114.09a	5.404a	0.371a	6.9a
28 DAS					
16/8	11.0b	207.31de	8.502de	0.389e	4.6b
24/8	10.6b	247.30cd	9.985cd	0.516cd	5.2ab
32/8	13.3a	289.95ab	13.084b	0.638b	4.9ab
20/10	9.0c	202.57e	6.884e	0.348e	5.1ab
30/10	10.2b	223.72de	8.272de	0.431de	5.2a
40/10	11.4b	269.72bc	11.060c	0.548bc	5.0ab
CL	12.8a	326.50a	15.664a	0.803a	5.1ab

<sup>Z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05

Table 1-2. Number of leaves, leaf area, shoot fresh and dry weights, and percentage of dry matter of leaf lettuces grown under various lighting cycle conditions.

	No. of leaves	Leaf area (cm <sup>2</sup> )	Shoot fresh weight (g)	Shoot dry weight (g)	Percentage of dry matter (%)
14 DAS					
16/8	6.6b <sup>Z</sup>	39.02c	0.926d	0.055d	5.9b
24/8	5.6c	39.07c	1.101c	0.065cd	5.9b
32/8	7.0b	54.33b	1.596b	0.107b	6.7a
20/10	5.0d	17.01e	0.488e	0.033e	6.8a
30/10	6.0c	27.00d	0.816d	0.055d	6.8a
40/10	6.6b	38.43c	1.150c	0.073c	6.3ab
CL	8.4a	65.60a	2.078a	0.131a	6.3ab
21 DAS					
16/8	14.4b	133.44bc	4.315c	0.241c	5.6ab
24/8	14.0b	126.93cd	4.667c	0.252c	5.4b
32/8	13.6b	139.72bc	5.613b	0.317b	5.7ab
20/10	11.6c	74.72e	2.478d	0.156d	6.3a
30/10	13.4b	118.48d	3.979c	0.242c	6.1ab
40/10	13.4b	146.53b	5.414b	0.292b	5.4b
CL	16.4a	174.90a	8.083a	0.492a	6.2a
28 DAS					
16/8	22.0cd	365.89b	12.897cd	0.702c	5.4ab
24/8	23.0bc	397.33ab	13.772c	0.625cd	4.5c
32/8	23.3bc	431.16a	15.982b	0.928a	5.8a
20/10	19.4e	262.85c	7.761e	0.398e	5.1bc
30/10	21.2d	364.95b	11.346d	0.569d	5.0bc
40/10	24.0ab	431.18a	14.415c	0.719bc	5.0bc
CL	25.0a	429.17a	17.663a	0.833ab	4.7c

<sup>Z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05

## **Plant growth of Chinese cabbages and pak-chois**

In Chinese cabbages and pak-chois, shoot and dry weight, LUE and CGR were greatest in treatment CL until 14 DAS. Those of Chinese cabbages were greatest in treatment CL until 21 DAS, while those in treatment 40/10 were greatest at 28 DAS. Those of pak-chois, on the other hand, were greatest in treatments 40/10 and 32/8 at 21 and 28 DAS (Figs. 1-4, 1-5, and 1-6). Growth responses of Chinese cabbages and pak-chois to ELCs were similar to those of lettuces. Shoot fresh weight and leaf area showed a similar trend to shoot dry weight (Tables 1-3 and 1-4). In both plants, as the ratio of photoperiod to lighting cycle increased, leaf area, shoot fresh and dry weights, LUE, and CGR increased except treatment CL.

Various studies had conducted to promote the growth of Chinese cabbage by controlling soil composition (Stapleton et al., 1985), light intensity (Xu et al., 2009). However, few studies on the effects of photoperiod to growth of Chinese cabbage and pak-chois have been reported.

Extended lighting cycles can be applied to promote the growth of Chinese cabbages during its transplant production, e.g., treatments CL and 40/10 up to 21 and 28 DAS, respectively. And 32/8-h lighting cycle can be proper to production of pak-chois at least up to until 28 DAS.

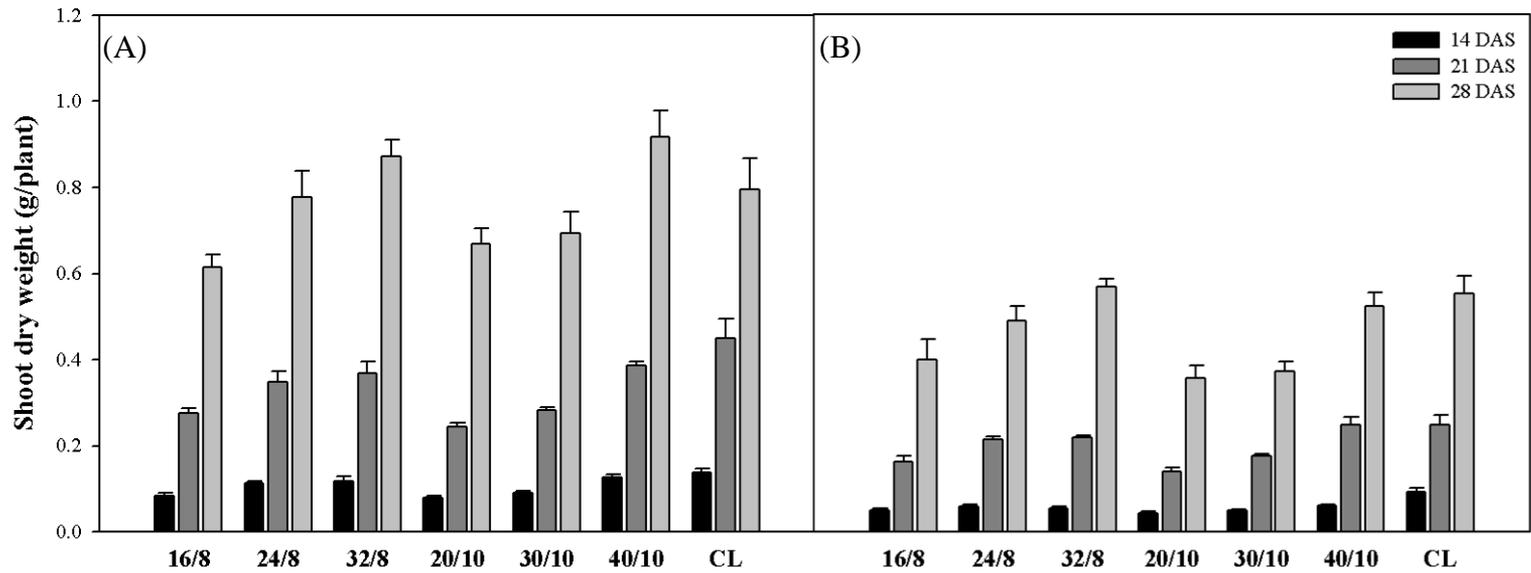


Fig. 1-4. Shoot dry weight of Chinese cabbages (A) and pak-chois (B) grown for 28 days under various lighting cycle conditions.

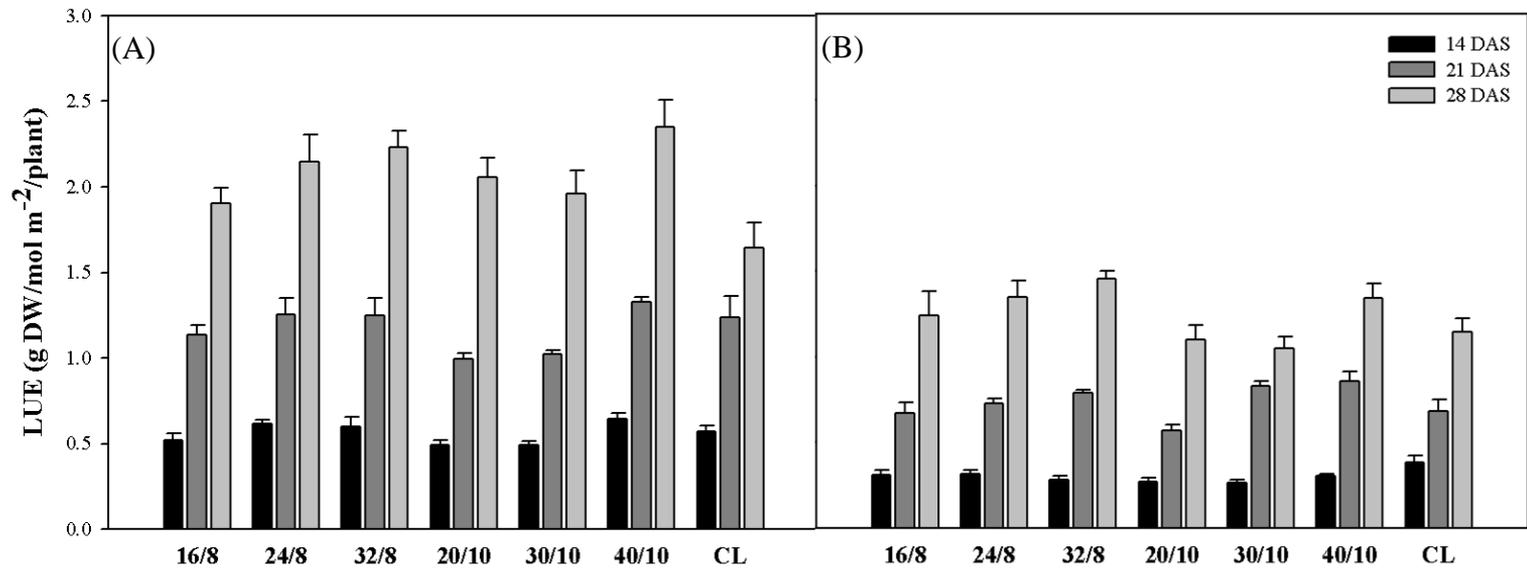


Fig. 1-5. Light use efficiency (LUE) of Chinese cabbages (A) and pak-chois (B) grown for 28 days under various lighting cycle conditions.

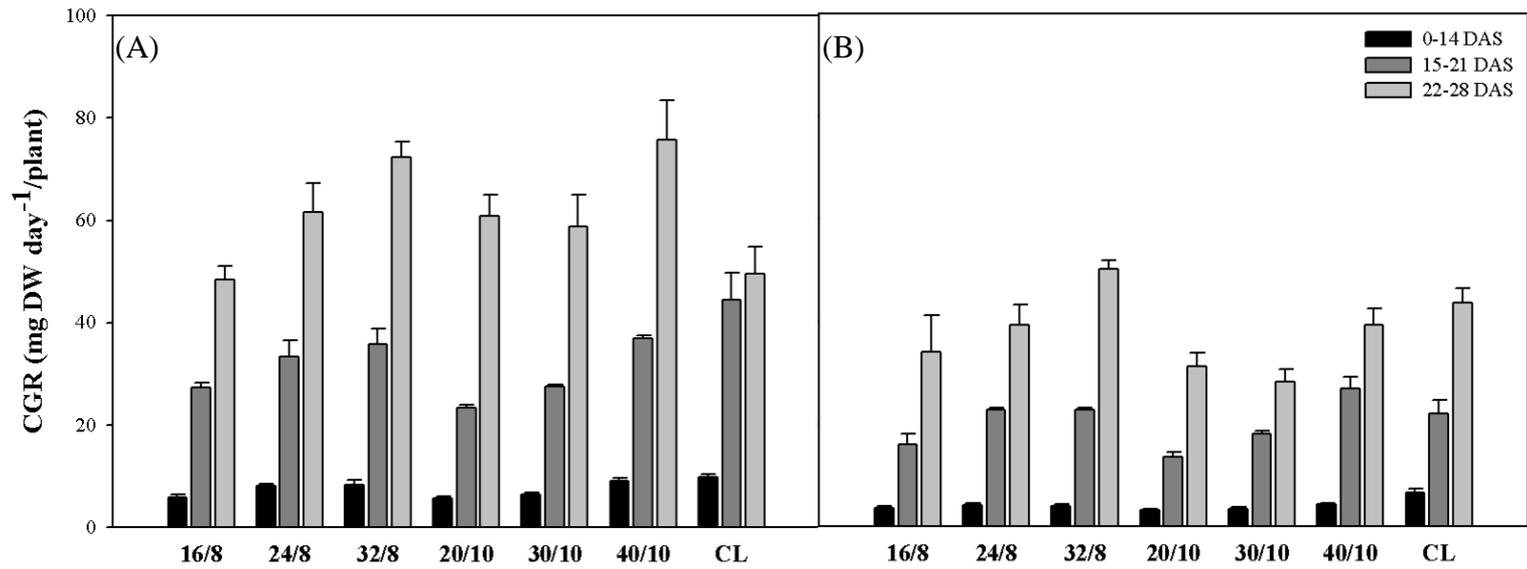


Fig. 1-6. Crop growth rate (CGR) of Chinese cabbages (A) and pak-chois (B) grown for 28 days under various lighting cycle conditions.

Table 1-3. Number of leaves, leaf area, shoot fresh and dry weights, and percentage of dry matter of Chinese cabbages grown under various lighting cycle conditions.

	No. of leaves	Leaf area (cm <sup>2</sup> )	Shoot fresh weight (g)	Shoot dry weight (g)	Percentage of dry matter (%)
14 DAS					
16/8	4.4ab <sup>Z</sup>	27.66cd	1.509cd	0.084c	5.5d
24/8	5.0a	31.63bc	1.729bc	0.114b	6.6ab
32/8	5.0a	35.31b	1.839b	0.118ab	6.4bc
20/10	4.0b	23.20d	1.135e	0.081c	7.1a
30/10	4.6ab	26.87cd	1.321de	0.091c	6.9ab
40/10	5.0a	41.18a	2.170a	0.128ab	5.9cd
CL	4.6ab	36.13ab	2.161a	0.138a	6.4bc
21 DAS					
16/8	7.8a	87.55b	5.156b	0.275d	5.4c
24/8	7.8a	97.98ab	6.455a	0.348bc	5.5bc
32/8	7.0a	97.90ab	6.199a	0.368b	5.9abc
20/10	7.4a	82.88b	4.145b	0.244d	5.9abc
30/10	7.6a	81.87b	4.537b	0.283cd	6.3ab
40/10	8.0a	115.05a	6.593a	0.386ab	5.9abc
CL	7.0a	109.02a	6.894a	0.449a	6.5a
28 DAS					
16/8	9.8a	197.04c	12.220c	0.615c	5.0a
24/8	9.8a	239.66b	14.551ab	0.778ab	5.3a
32/8	9.7a	261.31ab	15.744a	0.873a	5.5a
20/10	10.0a	226.12bc	12.261c	0.670bc	5.5a
30/10	9.4a	227.11bc	12.727bc	0.695bc	5.5a
40/10	10.4a	287.17a	16.421a	0.917a	5.6a
CL	10.2a	247.12b	15.722a	0.796ab	5.0a

<sup>Z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05

Table 1-4. Number of leaves, leaf area, shoot fresh and dry weights, and percentage of dry matter of pak-chois grown under various lighting cycle conditions.

	No. of leaves	Leaf area (cm <sup>2</sup> )	Shoot fresh weight (g)	Shoot dry weight (g)	Percentage of dry matter (%)
14 DAS					
16/8	4.2b <sup>Z</sup>	13.94bc	0.737bc	0.050bc	6.8b
24/8	4.2b	13.13bc	0.716bc	0.058bc	8.2a
32/8	4.6ab	14.58bc	0.796bc	0.055bc	7.0b
20/10	4.2b	11.02c	0.601c	0.044c	7.3b
30/10	4.4ab	12.89c	0.662bc	0.049bc	7.4b
40/10	4.6ab	17.13b	0.871b	0.060b	7.0b
CL	5.0a	22.99a	1.258a	0.093a	7.5b
21 DAS					
16/8	6.8bc	47.01bc	2.509cd	0.162c	6.4a
24/8	5.8d	47.60bc	2.963bc	0.215ab	7.3a
32/8	7.8a	54.83b	3.342ab	0.218a	6.5a
20/10	7.6ab	42.09c	2.168d	0.140c	6.4a
30/10	6.4cd	48.49bc	2.606cd	0.175bc	6.8a
40/10	6.8bc	65.32a	3.610a	0.249a	6.9a
CL	6.2cd	51.28bc	3.648a	0.247a	6.9a
28 DAS					
16/8	7.4b	103.96bc	7.370b	0.401bc	5.4b
24/8	9.6a	116.35abc	9.424a	0.488ab	5.2b
32/8	9.5a	133.43a	9.824a	0.570a	5.8ab
20/10	7.4b	102.06c	5.872b	0.358c	6.1a
30/10	8.0b	106.91bc	6.821b	0.372c	5.5ab
40/10	7.4b	127.65ab	9.115a	0.524a	5.8ab
CL	8.6ab	125.59abc	10.390a	0.553a	5.3b

<sup>Z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05

## **Plant growth of hot peppers and tomatoes**

In hot peppers, number of leaves, stem diameter, shoot fresh and dry weights were greatest in treatment CL, however, LUE and CGR in treatment 32/8 were greatest at 14 DAS (Figs. 1-8 and 1-9, and Table 1-5). Tamamoto et al. (2008) reported that CL treatment showed negative effects on growth of peppers. Similarly, in the present study, LUE and leaf area of hot peppers were smallest in treatment CL at 28 DAS (Fig. 1-8 and Table 1-5). At 28 DAS, number of leaves, leaf area, plant height, stem diameter, shoot fresh and dry weights, LUE and CGR were greatest in treatment 40/10. All the growth parameters investigated, LUC, and CGR did not depend on the ratio of photoperiod to lighting cycle (Figs. 1-7, 1-8, and 1-9, and Table 5). Considering that shoot fresh and dry weights, LUE, and leaf area, the 40/10-h lighting cycle was thought to be proper to promote growth of peppers seedling production at least up to 28 DAS. Growth of hot pepper 'Perfection Pimiento' was enhanced as the photoperiod lengthened (Cochran, 1942; Nilwik, 1981).

In tomatoes, shoot fresh and dry weights in the ELC treatments including the CL were greater than or similar to those in treatment 16/8 (Fig. 1-7 and Table 1-5), while chlorosis and necrosis on leaves were observed in treatment CL at 14 DAS (data not shown). All the growth parameters investigated were greatest in treatment 32/8 at 14 DAS, but, after then growth of tomatoes became retarded as

photoperiod in the ELCs increased resulting in chlorosis and necrosis on leaves in treatments 32/8, 30/10, 40/10 and CL at 21 DAS. Especially, growth of tomatoes grown in treatment CL had completely stopped and the plants were completely dried out. Tomatoes in treatment 20/10 did not show any negative symptoms until 28 DAS and their growth were significantly promoted comparing with other treatments (Figs. 1-7, 1-8, and 1-9, and Table 6).

ELCs e.g., treatments 16/8 and 40/10 up to 21 and 28 DAS, respectively can be applied to promote the growth of hot peppers during transplant production. The 20/10-h lighting cycle can be proper to production of tomato seedlings at least up to 28 DAS.

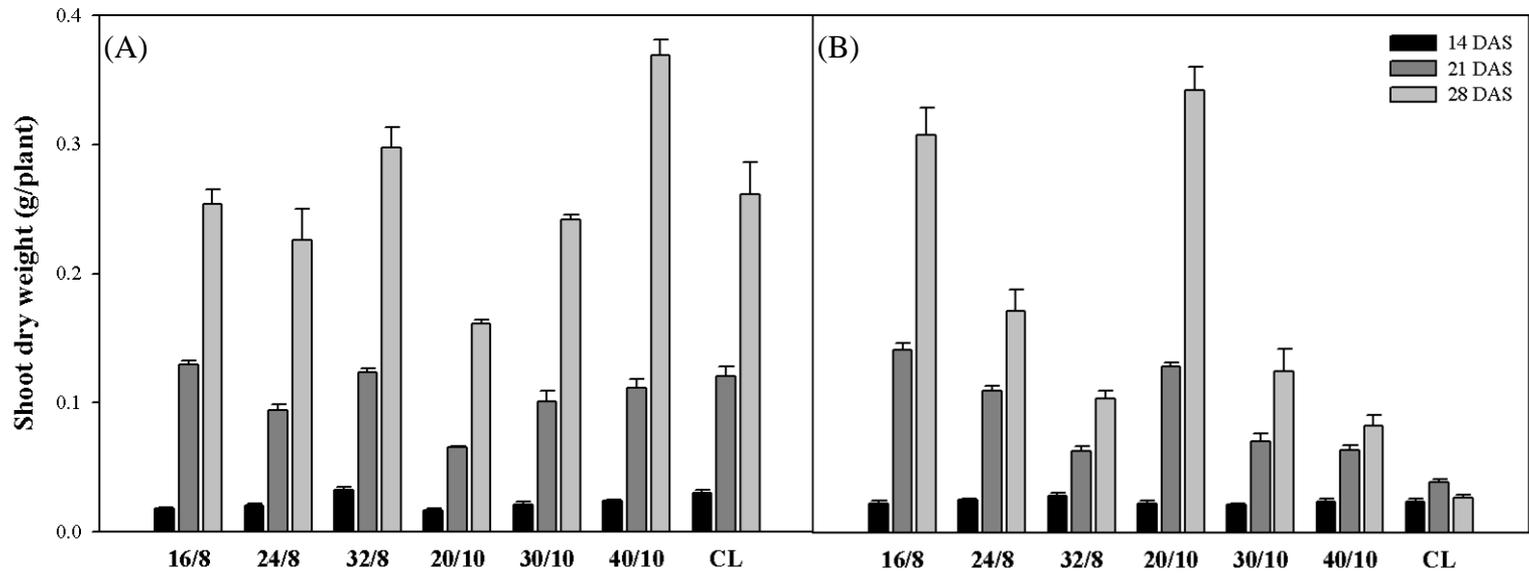


Fig. 1-7. Shoot dry weight of hot peppers (A) and tomatoes (B) grown for 28 days under various lighting cycle conditions.

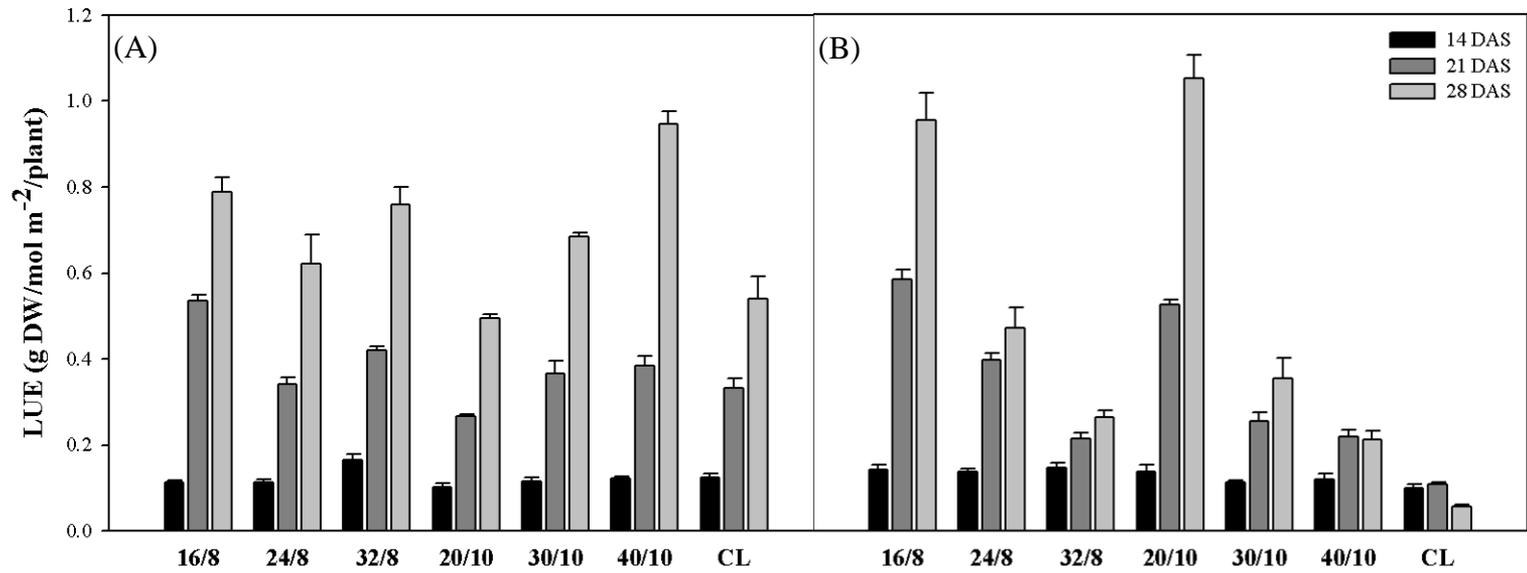


Fig. 1-8. Light use efficiency (LUE) of hot peppers (A) and tomatoes (B) grown for 28 days under various lighting cycle conditions.

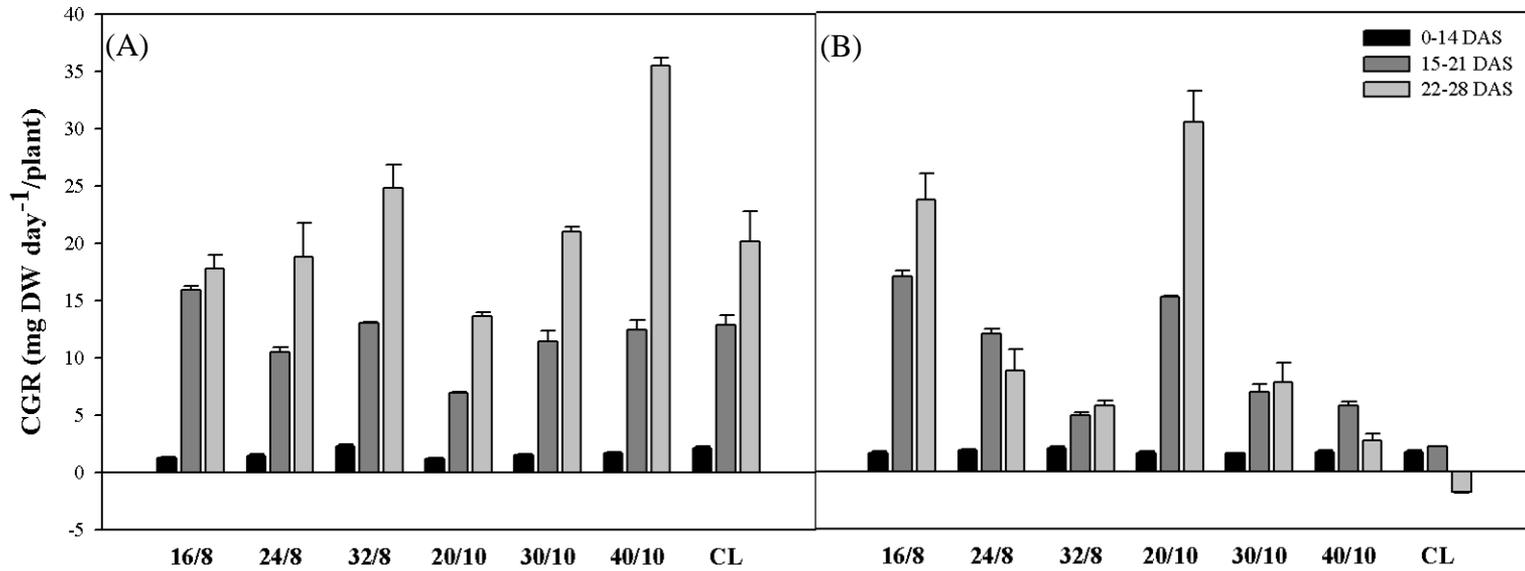


Fig. 1-9. Crop growth rate (CGR) of hot peppers (A) and tomatoes (B) grown for 28 days under various lighting cycle conditions.

Table 1-5. Number of leaves, leaf area, plant height, hypocotyl length, stem diameter, shoot fresh and dry weights, and percentage of dry matter of hot peppers grown under various lighting cycle conditions.

	No. of leaves	Leaf area (cm <sup>2</sup> )	Plant height (mm)	Hypocotyl length (mm)	Stem diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Percentage of dry matter (%)
14 DAS								
16/8	1.8abc <sup>Z</sup>	1.57bc	38.2ab	20.4ab	11.9b	0.244b	0.018c	7.5c
24/8	0.8c	0.95c	36.6ab	21.8a	10.8cd	0.228bc	0.021bc	9.1b
32/8	2.6ab	3.19a	40.6a	20.8ab	12.0b	0.271ab	0.032a	12.0a
20/10	0.8c	0.59c	31.6c	19.4bc	10.6d	0.183c	0.017c	9.1b
30/10	1.4bc	1.38bc	34.6bc	20.2abc	11.0bcd	0.235b	0.021bc	9.1b
40/10	1.6abc	1.56bc	36.0b	18.2c	11.8bc	0.259ab	0.024b	9.4b
CL	2.8a	2.56ab	36.8ab	18.2c	13.2a	0.302a	0.030a	10.0b
21 DAS								
16/8	8.2a	29.97a	87.0a	24.4a	25.0a	1.255a	0.130a	10.3bc
24/8	7.0c	18.05c	73.0c	20.2b	20.4cd	0.952cd	0.094d	9.9c
32/8	7.6abc	22.44b	75.2c	21.0b	22.4bc	1.142ab	0.123ab	10.9ab
20/10	5.6d	13.63d	64.8d	20.8b	17.9e	0.734e	0.065e	8.9d
30/10	7.2bc	20.90bc	74.2c	20.6b	19.2de	0.910d	0.101cd	11.2a
40/10	6.8c	22.66b	73.8c	21.0b	22.3bc	1.088bc	0.112bc	10.3bc
CL	8.0ab	19.35c	79.4b	20.2b	23.0b	1.042bcd	0.120ab	11.6a
28 DAS								
16/8	11.2bcd	67.26b	134.8a	2.34a	34.5a	2.822b	0.254bc	9.0c
24/8	11.0bcd	52.66de	122.8b	1.98c	30.3bc	2.443b	0.226c	9.2c
32/8	11.6abc	55.93cd	125.6b	2.18abc	33.6a	2.844b	0.297b	10.5ab
20/10	9.4d	41.08f	112.0c	2.06bc	28.6c	1.879c	0.161d	8.6c
30/10	10.0cd	64.07bc	125.8b	2.24ab	28.8c	2.462b	0.242c	9.8b
40/10	13.4a	80.21a	133.2a	2.00c	35.1a	3.662a	0.369a	10.1b
CL	12.4ab	44.13ef	113.8c	1.94c	32.4ab	2.419b	0.261bc	10.8a

<sup>Z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05

Table 1-5. Number of leaves, leaf area, plant height, hypocotyl length, stem diameter, shoot fresh and dry weights, and percentage of dry matter of tomatoes grown under various lighting cycle conditions.

	No. of leaves	Leaf area (cm <sup>2</sup> )	Plant height (mm)	Hypocotyl length (mm)	Stem diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Percentage of dry matter (%)
14 DAS								
16/8	2.4ab <sup>Z</sup>	3.44ab	80.0ab	40.4bc	14.5ab	0.314a	0.023ab	7.2b
24/8	2.4ab	3.80a	83.2a	40.8b	14.3ab	0.349a	0.026ab	7.3b
32/8	2.8a	4.14a	84.2a	38.8bc	15.1a	0.364a	0.029a	7.9a
20/10	1.6c	1.97c	80.6ab	46.2a	14.0ab	0.335a	0.023ab	6.7c
30/10	2.0bc	2.08c	77.0abc	41.2b	13.2b	0.303a	0.022b	7.3b
40/10	2.4ab	2.44bc	75.0bc	37.2c	14.1ab	0.304a	0.024ab	7.8a
CL	2.4ab	2.97abc	70.8c	29.2d	15.1a	0.301a	0.024ab	8.1a
21 DAS								
16/8	5.8a	25.69a	159.4a	44.6a	25.9a	1.952a	0.142a	7.3c
24/8	5.2b	24.90a	145.4b	42.6a	24.7ab	1.575c	0.110c	7.0c
32/8	4.8bcd	5.14d	116.4d	36.4bc	18.8d	0.743e	0.063d	8.5b
20/10	4.6cd	20.84b	142.6b	44.4a	23.4b	1.777b	0.129b	7.3c
30/10	4.4d	13.52c	121.8cd	37.6b	19.4cd	0.967d	0.071d	7.3c
40/10	5.0bc	11.43c	126.4c	32.8cd	20.8c	0.875de	0.064d	7.3c
CL	3.0E	-	94.4e	29.0d	15.7e	0.371f	0.039e	10.6a
28 DAS								
16/8	6.6bcd	71.90b	215.2b	38.6a	31.3b	4.194b	0.308a	7.3bc
24/8	7.4ab	37.02c	187.0c	36.4a	25.3c	2.365c	0.171b	7.3bc
32/8	5.8d	8.75de	153.8d	36.8a	19.8d	1.171de	0.104c	8.8b
20/10	7.8a	89.35a	237.0a	39.6a	35.4a	5.056a	0.342a	6.8c
30/10	6.0cd	19.24d	182.6c	37.6a	25.9c	1.674d	0.125c	7.4bc
40/10	6.8bc	10.26de	161.0d	35.8a	23.0c	1.113e	0.083c	7.5bc
CL	3.6e	-	94.0e	29.0b	15.5e	0.217f	0.027d	13.0a

<sup>Z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05

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## **CHAPTER 2**

### **Insertion of Dark Period to Avoid Growth Inhibition Caused by Continuous Lighting in Tomato**

#### **INTRODUCTION**

Plant growth is hampered by short-day conditions especially during autumn and winter (Murage et al., 1996). This problem can be overcome by the use of artificial lighting in greenhouses and other plant production systems. However, prolonging the photoperiod to near or continuous light without dark period can cause injury to susceptible plants such as chrysanthemum (Warrington and Norton, 1991), eggplant (Murage, 1996), potato (Tibbitts, et al., 1990; Cao and Tibbitts, 1992), sweet pepper (Nilwik, 1981), tomato (Arthur et al., 1930), and so on. Among them, tomatoes grown under continuous lighting (CL) condition showed various negative symptoms e.g., chlorosis (Withrow and Withrow, 1949; Demer and Gosselin, 2000), necrosis (Hillman, 1956), decrease in chlorophyll content (Dorais et al., 1995; Jensen and Veiershow, 1998), and increases in

photo-oxidative pressure (Demer and Gosselin, 2002). Demer and Gosselin, (2002) reported that tomato plants show the more severe CL-induced injuries comparing to peppers, which correlates with the lower carotene and xanthophyll contents. Similarly, compared to peppers, eggplant plants have the lower reactive oxygen species (ROS)-detoxifying enzyme activities, correlating with the absence of CL-induced injury (Murage and Masuda, 1997).

CL-induced injury can be delayed by provided the periodicity of environment conditions in tomato. For instance, growth inhibition caused by CL (e.g., chlorosis and/or necrosis) was not observed under alternating air temperature condition with continuous lighting (Ohyama et al., 2005). The best way to avoid the CL-induced injuries has been thought to be to supply proper photoperiods. Under natural photoperiod condition provided combination of photoperiod and dark period, chlorosis and/or necrosis were rarely happened. Objective of this study were to avoid CL-induced growth inhibition of tomato plants, that showed the most severe CL-induced injuries in the first chapter, by insertion of dark period in the middle of CL.

## MATERIALS AND METHODS

### Plant materials and culture conditions

In the first experiment, tomato “Momotaro” (Takii Seed Co., Japan) plants were grown under six lighting cycle conditions; CL to 16/8 h lighting cycle 6, 8, 10, and 12 days after sowing (treatment DT6, DT8, DT10, and DT12, respectively), and CL (treatment CL) and 16/8 h lighting cycle unchanged throughout the experiment (as control). In the second experiment, tomato plants were grown under CL condition for 10 days, and six different lengths of dark period (8, 16, 24, 32, 40, and 48 h) were inserted (treatments LD8, LD16, LD24, LD32, LD40, and LD48, respectively), or grown under CL (treatment CL) and 16/8-h lighting cycle (as control) conditions unchanged throughout the experiment.

Photosynthetic photon flux at empty shelves during photoperiod was  $187 \pm 13 \mu\text{mol m}^{-2} \text{s}^{-1}$  and air temperature was maintained at 28/25°C during photo-/dark periods. The seedlings were subirrigated for 20 min once a day with the standard nutrient solution developed by National Horticultural Experiment Station, Japan. Data were analyzed using SAS 9.1 version (SAS Inst. Inc., Cary, NC, USA) for Duncan’s multiple range test (DMRT) at  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

### **Insertion Timing of Dark Period to Avoid CL-induced Injuries**

Shoot dry weight was greatest in treatment CL at 10 DAS (Fig. 2-1). In the same manner, leaf area, plant height, stem diameter, and shoot fresh weight were greatest in treatment CL until 10 DAS (data not shown). Number of leaves, leaf area, shoot fresh and dry weights were smallest in treatment CTRL (16/8-h lighting cycle) at 10 DAS. Prolonging the photoperiod increases the hours that photosynthetic organisms can fix CO<sub>2</sub> and translate into more biomass, and cultivation under CL condition enhances to some extent the growth of *Arabidopsis* (Lepisto et al., 2009), lettuces (Gaudeau et al., 1994), potatoes (Wheeler and Tibbitts, 1986), and roses (Pettersen et al., 2007).

By contrast, number of leaves, leaf area, plant height, stem diameter, shoot fresh and dry weights were smallest in treatment CL while those in treatment DT6 were greatest at 20 DAS (Table 2-1). Chlorosis and necrosis on leaves were observed in treatment CL at 20 DAS (Fig. 2-2) and growth parameters in all of dark period insertion treatments were greater than those in treatment. Even shoot dry weight in three dark period insertion treatments (DT6, DT8, and DT10) was greater than that in treatment CTRL (Fig. 2-1).

Withrow and Withrow (1949), Hillman (1956), and Demer and Gosselin (2000) had reported the CL-induced growth inhibitions that were generally not observed in the dark or under natural photoperiodic conditions in tomatoes (Foyer et al., 1994). Demers et al. (1998) claimed that dark periods were necessary in order to prevent leaf injuries such as chlorosis and necrosis in tomatoes. The results from this experiment indicate that CL-induced growth inhibition can be avoided by inserting dark periods in the middle of CL at earlier stages.

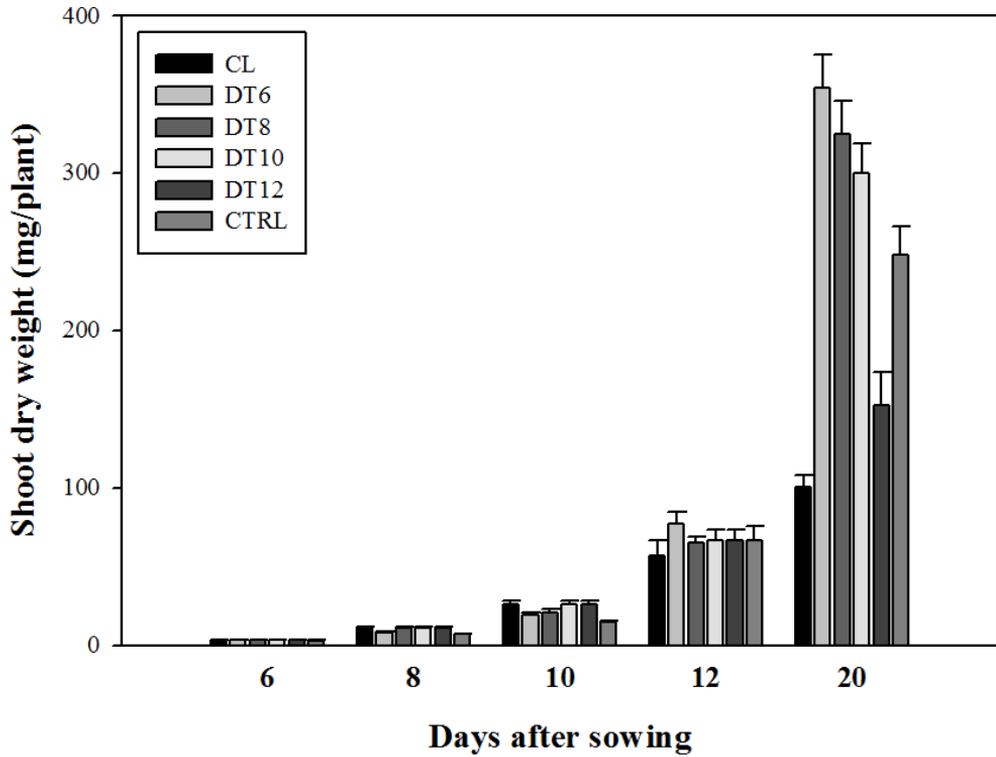


Fig. 2-1. Shoot dry weight of tomatoes grown for 20 days as affected by insertion timing of dark periods in the middle of continuous lighting

Table 2-1. Number of leaves, leaf area, plant height, hypocotyl length, and stem diameter of tomatoes 20 days after sowing as affected by insertion timing of dark period in the middle of continuous lighting.

Treatment code	No. of leaves	Leaf area (cm <sup>2</sup> )	Plant height (mm)	Hypocotyl length (mm)	Stem diameter (mm)
CL	4.3 c <sup>Z</sup>	16.29 c	123.2 c	34.8 bc	2.53 e
DT6	5.5 a	86.32 a	162.5 a	37.2 b	3.84 a
DT8	5.2 ab	72.35 b	164.7 a	31.7 c	3.60 b
DT10	4.8 bc	66.19 b	143.3 b	32.7 c	3.35 c
DT12	4.5 c	36.04 c	156.3 a	34.2 bc	2.78 d
CTRL	4.8 bc	61.96 b	145.8 b	41.5 a	3.36 c

<sup>Z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05

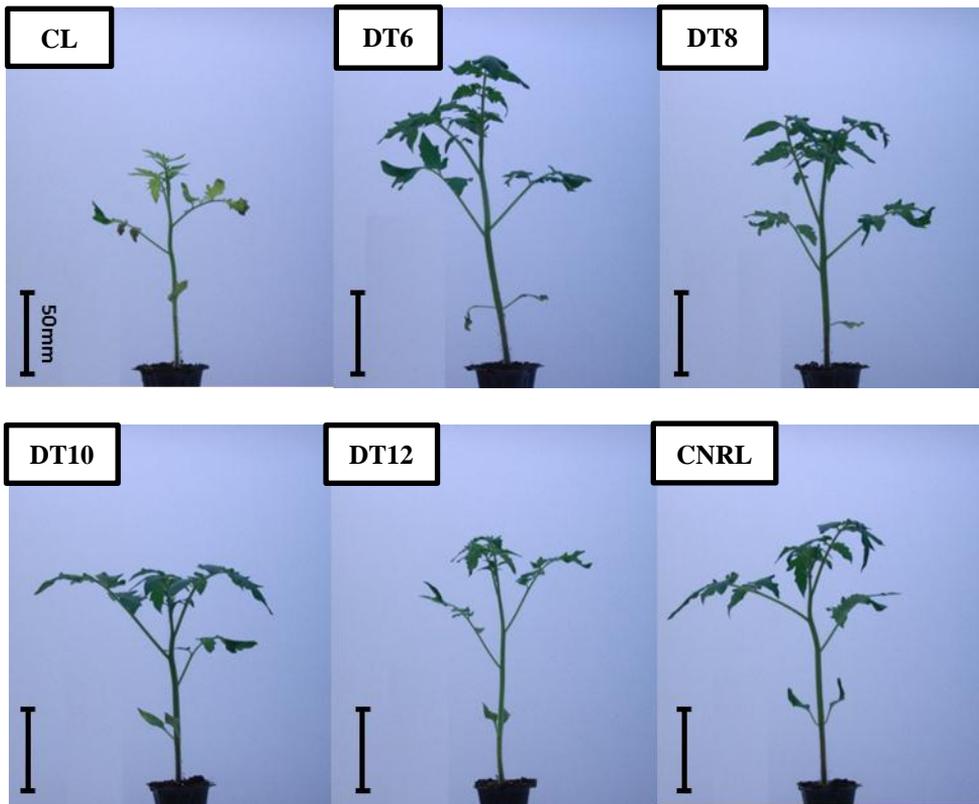


Fig. 2-2. Tomato plants grown under various photoperiodic conditions for 20 days with different insertion timing of dark periods in the middle of continuous lighting.

### **Length of Dark Period Inserted Necessary to Avoid CL-induced Injuries**

Shoot fresh and dry weights, number of leaves, leaf area, plant height, and stem diameter were greatest in treatment LD16 at 14 DAS (Fig. 2-3 and Table 2-2). Those decreased as the length of dark period inserted increased. Murage et al. (1996) reported that, although the level of leaf chlorophyll was highest in eggplants grown with 12-h daily dark period, eggplants grown with fewer than 9-h of daily dark periods had greater biomass accumulation. Results from this experiment indicated that growth parameters increased as the ratio of photoperiod to growing period increased at earlier stage.

At 17 DAS, however, all the growth parameters investigated was greatest in treatment CTRL, while those in treatment CL were smallest (Fig. 2-3 and Table 2-2). Shoot dry weight in all the dark-period-inserted treatments was greater than that in treatment CL. Shoot dry weight in treatment LD48, where showed the smallest growth until 14 DAS, were even greater than that in treatment CL. Effectiveness of avoidance of CL-induced injuries did not change by various length of dark periods inserted from 8 up to 48 hours. The results from this experiment indicate that increasing insertion frequency of dark period was more efficient than extending the length of dark period to avoid CL-induced growth inhibition.

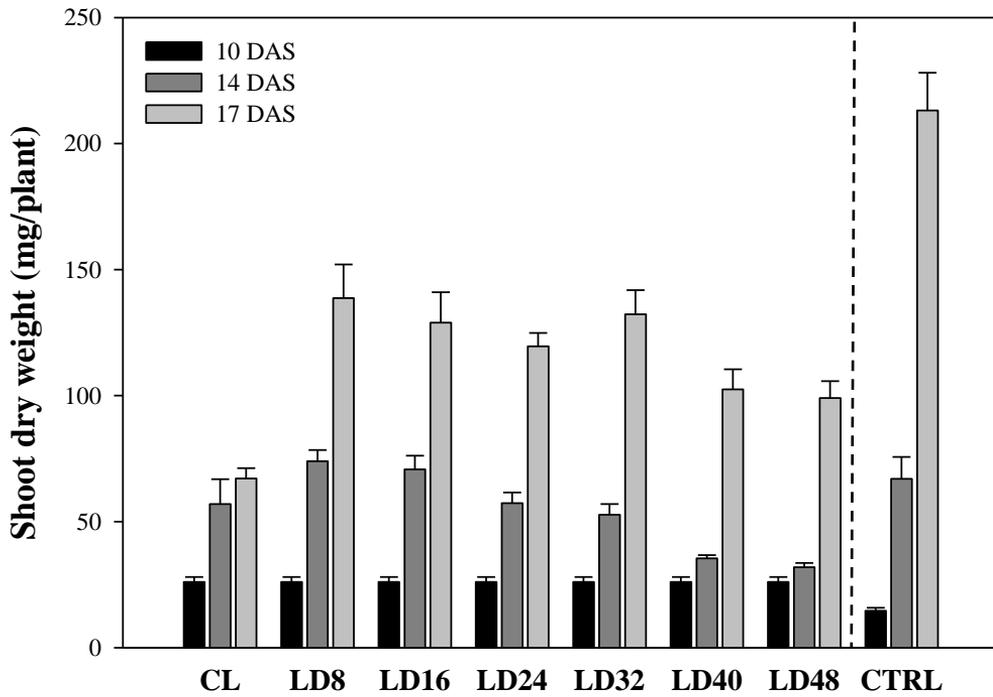


Fig. 2-3. Shoot dry weight of tomatoes grown for 17 days as affected by length of dark period inserted at starting of 11 DAS.

Table 2-2. Number of leaves, leaf area, plant height, and stem diameter of tomatoes 14 days after sowing as affected by length of dark period inserted at starting of 11 DAS.

Treatment code	No. of leaves	Leaf area (cm <sup>2</sup> )	Plant height (mm)	Stem diameter (mm)
CL	2.7bc <sup>z</sup>	10.27b	74.5ab	20.6cd
LD8	3.2ab	16.33a	80.3ab	22.3ab
LD16	3.5a	14.42a	83.2a	23.0a
LD24	3.2ab	11.20b	78.5ab	20.5bc
LD32	2.8bc	9.98b	74.7ab	20.3bcd
LD40	2.0de	4.94c	56.7cd	18.9cd
LD48	2.0de	4.34c	62.7c	18.0d
CTRL	2.5cd	15.25a	72.3b	20.6bc

<sup>z</sup>Mean separation within columns in each treatment by DMRT at P < 0.05



Fig. 2-4. Tomato plants grown for 14 (upper) and 17 (lower) under various photoperiodic conditions with different lengths of dark period after 11 days of continuous lighting.

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## CONCLUSION

Growth response to ELC varied among six tested vegetables. ELCs including CL promoted vegetable growth resulting in greater light use efficiency at the earlier stages, while the ELC-induced injuries and/or growth inhibition was observed at the later stages. Starting point and sensitivity of growth inhibition were earliest and most severe in tomatoes, followed by hot peppers, pak-chois, Chinese cabbages, leaf lettuces, and head lettuces.

Growth inhibition caused by ELC or CL could be avoided by insertion of dark periods. Effectiveness of the avoidance decreased as insertion of dark period was delayed, but the lengths of dark period inserted from 8 up to 48 h did not change the effectiveness. The ELC or CL with insertion of dark periods not necessarily longer than 8 h could be applied for rapid and efficient production of leafy and fruit vegetables and/or their transplants in closed plant production systems using artificial lights.

## ABSTRACT IN KOREAN

본 연구에서는 폐쇄형 식물생산 시스템에 적용 가능한 extended lighting cycle (ELC)이라는 새로운 광주기를 제안하였다. 제 1 장에서는 연속광을 포함하는 ELC(암기 8 시간에 명기 16, 24, 32 시간, 암기 10 시간에 명기 20, 30, 40 시간을 조합)조건이 결구상추, 잎상추, 배추, 청경채, 고추, 토마토 등 6 가지 주요 채소의 생장에 미치는 효과를 구명하였다. 광주기 대비 명기의 비율(명기율)이 커질수록, 생육 초기에는 성장량과 광이용 효율이 증가하였지만 일정 시기 이후에는 생육 저해 현상이 나타났다. 토마토의 경우 파종 후 14 일부터 연속광 조건에서 생육 저해 및 잎의 황백화가 발생하였고, 이러한 현상은 명기율이 높은 ELC 처리일수록 더 빠르고 심하게 진행되었다. 또한 이와 같은 생육 저해 현상의 발생 시점과 피해 정도는 공시 채소의 종류에 따라 달라서, 토마토, 고추, 청경채, 배추, 잎상추, 결구상추 순으로 민감하였다.

제 2 장에서는, 제 1 장의 연구에서 연속광을 포함한 ELC 조건에 가장 민감하게 반응하여 이른 시기부터 심한 생육 저해 현상을 나타낸 토마토를 대상으로 연속광 중에, 혹은 일정 기간의 연속광 조사 이후에 암기를 삽입함으로써 연속광에 의한 생육 저해 현상을 회피할 수 있는지 연구하였다. 연속광 조사 이후에 16/8-h 광주기로 전환하는 시점(파종 후 6, 8, 10, 12 일)을 달리한 광조사 조건과, 동일한 시점(파종 후 10 일 이후)에 삽입한 암기의 길이(8, 16, 24, 32, 40, 48 시간)를 달리한 조건에서 토마토 유묘를 생육시킨

결과, 연속광에 의한 생육 저해 현상은 모든 암기 삽입 처리구에서 회피되는 것을 밝혀내었다. 연속광 조건에서 8h 의 암기를 포함하는 24h 광주기로 전환하는 시점이 늦을수록 생육 저해 현상의 회피 효과는 감소하였다. 또한 10 일간의 연속광 조사 이후에 암기를 삽입할 경우 8h 부터 48h 까지의 암기 증가는 생육 저해 현상 회피 효과의 정도에는 크게 영향을 미치지 않았다. 따라서 토마토 유묘의 생산 일수 단축 및 생육 촉진을 위해 연속광 혹은 ELC 조건을 적용하였을 경우 발생할 수 있는 생육 저해 현상은 연속광 사이에 수차례의 짧은 암기를 삽입함으로써 회피할 수 있음을 밝혀내었다.

본 결과를 통해, 인공광을 이용하는 폐쇄형 식물생산 시스템에서 수확일이 소요되는 엽채류의 생산 기간을 단축시키거나, 수 주 내에 생산되는 엽채류 및 과채류의 모를 효율적으로 생산하는데 ELC 또는 짧은 암기가 삽입되는 연속광 조건이 적용될 수 있음을 확인할 수 있었다.