Effects of Day Length and Air Temperature Fluctuation on the Occurrence of Leaf Browning in Sesame Seedlings Cultured in a Plant Factory under Artificial Light

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INTRODUCTION

Sesame (Sesamum indicum), one of the most important oil seed crops, is widely cultivated in Asia and Africa, especially in the high temperature regions (Anilakumar et al., 2010), and its seed is also utilized as a cooking ingredient around the world. The seeds contain the functional component sesamin, a type of lignan. Sesamin is reported to possess health benefits such as a cholesterol-lowering effect (Ogawa et al., 1995; Hirata et al., 1996), preventing high blood pressure and increasing vitamin E supply (Yamashita et al., 1992; Kamal-Eldin et al., 1995). Recently, sesamin has also been found in sesame leaves, and although the content is less than 1/5000 that in seeds (Hata et al., 2010), it has been found that cultivation using plastic pot filled with commercial soil-mix under continuous lighting at constant 28°C increases sesamin content in the leaves up to 71.5 μg g⁻¹ DW⁻¹, about 30 times compared to plants cultured under photoperiod of 12, 16 and 20-h with fluorescent lamp (Hata et al., 2012). Plant factories, especially the enclosed type using wholly artificial light, create the optimum cultivation conditions for year-round production of vegetables because all environmental factors can be managed precisely. Furthermore, plant factories are ideal for producing vegetables with enhanced functionality (such as high antioxidant activity), since “unnatural” environments (such as continuous lighting and minus value of difference between day and night temperature) can be provided. However, since managing and operating fully enclosed plant factories is expensive, it is necessary to cultivate high value crops (such as highly functional vegetables) to recoup the costs (Shimizu, 2014).

On the above evidence, we considered that sesame seedlings could become a new functional vegetable crop with high sesamin content in the leaves if cultivated in a plant factory under artificial light. Thus we tried to cultivate sesame plants hydroponically in plant factory under the environment of temperature (constant 28°C) and photoperiod (24-h) reported by Hata et al. (2012). However, we recognized that leaf browning was a problem in sesame seedlings cultured hydroponically in a plant factory, progressing from lower leaves to upper leaves (unpublished). Thus, it is necessary to establish the means to avoid the occurrence of leaf browning of sesame cultivated in plant factory. In this study, we investigated the effects of photoperiod and temperature regimes, based at 28°C of average temperature differences (06:00-18:00; 33°C, 18:00-06:00; 23°C), or with a 15°C difference (06:00-18:00; 35.5°C, 18:00-06:00; 20.5°C). Leaf browning was induced intensively by constant 28°C and avoided by air temperature fluctuation, especially with the 15°C difference. Shoot fresh weight and number of nodes were not significantly different among all the treatments. In experiment 2, plants were cultured under continuous lighting or a 12-h photoperiod either at a constant (28°C) or a fluctuating temperature (28°C with a 15°C difference). Leaf browning was induced by constant 28°C and avoided by air temperature fluctuation, regardless of photoperiod. Shoot fresh weight under a treatment with combination of temperature fluctuation with 15°C difference and continuous lighting was significantly greater than that under the other treatments. These results suggest that leaf browning of sesame seedlings is induced by constant air temperature and can be prevented by air temperature fluctuation.

Key words: constant air temperature, continuous lighting, diurnal air temperature regime, fluorescent light
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air temperature because of allowance to discuss about sesamin content in their leaves, on the occurrence and degree of leaf browning in sesame cultured in a plant factory under artificial light.

MATERIALS AND METHODS

Seeds of sesame cv. ‘Gomazou’ (commercial name ‘Gomazou’, Takii & Co., Ltd.) with high sesamin content in their leaves used in Hata et al. (2010; 2012), soaked in water for 1 d in darkness at 28°C, were sown on urethane cubes and grown for 10 d with 25% Enshi-shoho nutrient solution in the growth chamber (LHM-2000NC; Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan). The photoperiod was 12 h, the lighting source was a white fluorescent lamp (FHF32EN-H3; Mitsubishi Electric Lighting Co., Tokyo, Japan), and the photosynthetic photon flux density (PPFD) was 235 μmol m⁻² s⁻¹ at the surface of the urethane cubes. Air temperature was the same as each treatment of experiments. Twenty seedlings were transplanted onto a panel floating in 23 L plastic containers containing 17 L of 50% Enshi-shoho solution, and cultured under the environmental conditions designated for each treatment. Aeration to the nutrient solution was carried out by bubbling continuously with an air pump. Renewal and pH control of nutrient solution were not carried out.

[Experiment 1] Seeds were sown on September 7, 2015. Seedlings were cultured either at a constant 28°C of air temperature in the growth room of the plant factory in Kyoto Prefectural University, or fluctuating air temperature of 28°C with a 10°C difference (06:00–18:00; 33°C, 18.00–06:00; 23°C), or with a 15°C difference (06.00–18.00; 35.5°C, 18.00–06.00, 20.5°C) in the same growth chambers where seedlings were grown. The light condition for each treatment was continuous lighting with a same white fluorescent lamp as mentioned above and the PPFD of 235 μmol m⁻² s⁻¹ measured 2.5 cm above the transplanting panel. CO₂ concentrations in growth chambers of each treatment were not controlled and varied between 256 and 761 ppm in constant 28°C and between 289 and 610 ppm in 15°C difference. Humidity in growth chambers of each treatments were also not controlled and varied between 30.2 and 83.2% RH in constant 28°C and between 45.8 and 73.2% RH in 15°C difference. 40 plants were cultivated in each treatment, which was consisted of two replicate containers. At 3, 5, 7, 10 and 14 d after transplanting, all the seedlings cultivated in each treatment were scored (as per experiment 1) for degree of leaf browning. Thereafter, eight seedlings were selected randomly from each treatment. Four seedlings of the eight seedlings were chosen at random to measure shoot fresh weight and number of nodes.

RESULTS

[Experiment 1] At a constant 28°C, leaf browning of the sesame seedlings was extensive visually and as measured by the browning score, especially for the leaves of the 1st node (Figs 1 & 2). Leaf browning was observed as brown spots 1–2 mm in diameter, progressing as interveinal chlorosis to interveinal necrosis and browned leaf margins (like tip-burn). Under the regime with a temperature difference of 10°C, leaf browning of seedlings was suppressed and the scores for the leaves of the 1st and 3rd nodes were significantly lower than under the constant
28°C regime. Under the regime with a temperature difference of 15°C, leaf browning was suppressed markedly and no interveinal chlorosis or necrosis was observed and the scores for the leaves of all nodes were significantly lower than those under constant 28°C. Shoot fresh weight of the seedlings cultured under 2 fluctuating temperature regimes was significantly lower than that under constant 28°C, but the number of nodes did not differ significantly among three treatments (Table 1).

[Experiment 2] Leaf browning of seedlings was induced intensively when grown under constant temperature, irrespective of photoperiod (Figs 3 & 4, Table 2). As compared with constant temperature, fluctuating temperature significantly reduced occurrence of leaf browning of seedlings, irrespective of photoperiod. No effect of photoperiod on the score of leaf browning was observed in all the days of measurement. Shoot fresh weight of the seedlings cultured under a treatment with combination of temperature fluctuation and continuous lighting was significantly greater than that under the other treatments (Table 3). In all the seedlings cultured under continuous lighting, 5 nodes were present, and in those under 12 h lighting, 4 nodes were present.

Table 1 Effects of fluctuating air temperature of 28°C with 10°C or 15°C difference under continuous lighting on the shoot fresh weight and number of nodes of sesame seedlings cultivated for 14 d in plant factory.

| Treatments            | Shoot fresh weight (g) | Number of nodes |
|-----------------------|------------------------|-----------------|
| Constant 28°C         | 15.6 ± 7.8 a           | 4.6 ± 0.6 a     |
| 10°C difference       | 10.9 ± 2.5 b           | 4.5 ± 0.5 a     |
| 15°C difference       | 11.7 ± 1.8 b           | 4.8 ± 0.4 a     |

Values are means ± SD (n = 20). Means with different letters are significantly different at the 5% level by Tukey’s HSD in each column.

Table 2 Results of Mann-Whitney U test about scores of leaf browning occurred in the sesame plant cultivated in plant factory under environment combined with 2 air temperature regimes, including constant 28°C and fluctuating 28°C with 15°C difference, and 2 photoperiods, including 12-h photoperiod and continuous lighting.

| Days after transplanting | N   | Temperature difference | Photoperiod |
|--------------------------|-----|------------------------|-------------|
|                          | 3   | 5                      | 10          |
|                          | 40  | 24                     | ns          |
|                          | 32  | 16                     | ns          |
|                          | 14  | 8                      | ns          |

*ns and *** were represented as no significant and significant at P<0.001, respectively.
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Table 3  Effects of fluctuating air temperature of 28°C with 15°C difference under 12-h photoperiod or continuous lighting on the shoot fresh weight and number of nodes of sesame seedlings cultivated for 14 d in plant factory.

| Treatments                                    | Shoot fresh weight (g) | Number of nodes |
|-----------------------------------------------|------------------------|-----------------|
| Constant 28°C, 12-h photoperiod               | 7.0 ± 0.5 b⁷           | 4²              |
| Constant 28°C, continuous lighting            | 8.8 ± 1.5 b            | 5               |
| 15°C difference, 12-h photoperiod             | 6.3 ± 1.8 b            | 4               |
| 15°C difference, continuous lighting          | 13.2 ± 1.8 a           | 5               |

Multi-way ANOVA

| Temperature                                    | *         |
|-----------------------------------------------|-----------|
| Photoperiod                                    | **       |
| Temperature X photoperiod                      | **       |

*Values are means ± SD (n = 4). Means with different letters are significantly different at the 5% level by Tukey’s HSD.

On the other hand, it is also known that continuous lighting-induced leaf injury can be reduced or prevented by a diurnal fluctuation in air temperature, as seen in eggplant (Murage et al., 1997), potato (Tibbitts et al., 1990; Cao and Tibbitts, 1991; Cushman and Tibbitts, 1996), and tomato (Hillman, 1956; Ohyama et al., 2005; Sysoeva et al., 2012; Shibaeva and Sherudilo, 2015). Therefore, we carried out the preliminary experiment to investigate the effects of 5 and 10°C of air temperature fluctuation on suppression of leaf browning. As the result, the degree of leaf browning was suppressed significantly by fluctuating air temperature fluctuation of 28°C with 5 and 10°C difference compared to constant 28°C, although suppressive effect was not perfect.

In experiment 1 of the present study, consistent with the preliminary experiment mentioned above, leaf browning of sesame seedlings under continuous lighting was prevented by diurnal fluctuation of air temperature, especially with a 15°C temperature difference (Figs 1 & 2). However, in experiment 2, leaf browning of sesame seedlings also occurred under 12-h photoperiod at constant temperature (Fig. 3), and results of the statistical analysis showed that the only factor to consistently affect the occurrence of leaf injury was temperature (Table 2). The combined results of both experiments therefore suggest that leaf injury in sesame seedlings is induced by constant temperature rather than continuous lighting. These results are very important information to obtain healthy growth of sesame seedlings and lead to establishing the technique of producing sesame seedlings with high content of sesamin. And it is also beneficial that the shoot fresh weight of sesame seedlings cultured under 15°C difference and continuous lighting was highest among all the treatments, and the number of nodes was not affected (Table 3).

Although leaf browning scores for sesame seedlings cultured under fluctuating air temperature with 15°C difference increased 10 d after transplanting, the mean score remained <1 and only a few small brown spots were observed on the leaves. It might be that even this browning could be avoided by a greater air temperature fluctuation or greater average air temperature. Thus it is necessary to

DISCUSSION

Sesame is grown in hot climate with temperature around 27°C (Ayana, 2015) and requires high canopy induced by some factors, including waterlogging (Wei et al., 2018) and requires high canopy induction by some factors, including waterlogging (Wei et al., 2018). Thus, the average temperature 28/15°C was higher than that at 22/15°C. Thus, the average temperature 28°C in the experiments of this paper was reasonable and available for rapid growth of sesame seedlings cultivated in plant factory.

Occurring of leaf browning in sesame plants was induced by some factors, including waterlogging (Wei et al., 2013), Fe deficiency (Kannan, 1983), salt stress (Yahya, 1998). So, prior to carrying out the experiments in this study, we had carried out some experiments in order to screen the factors induced the leaf browning of sesame plants cultivated hydroponically in plant factory. It had been resulted in that intensive leaf browning occurred in any cases when sesame seedlings were cultivated under continuous lighting by hydroponic culture with no bubbling to nutrient solution, various Fe concentration in nutrient solution, under constant 100% RH, and by sheet culture in that roots were exposed to atmosphere at constant temperature of 28°C (the data not shown). Conversely, no leaf browning occurred at all, when sesame seedlings were cultivated hydroponically under continuous lighting with fluorescent lamp in the fully-shaded space with the silver plastic film (0.1 mm in thickness) equipped in the greenhouse in Experimental Farm of Kyoto Prefectural University, where air temperature was 31.6°C in average and varied between 24.0 and 43.1°C with diurnal temperature fluctuation between 4.6 and 15.5°C (the data not shown). Many studies have reported that leaf injuries such as chlorosis and necrosis occur during culture under continuous lighting in various species, including tomato (Arthur et al., 1930; Hillman, 1956; Cushman et al., 1995; Ohyama et al., 2005), eggplant (Murage et al., 1996), potato (Tibbitts et al., 1990; Cao and Tibbitts, 1991), and radish (Craker et al., 1983; Warrington and Norton, 1991).

On the other hand, it is also known that continuous lighting-induced leaf injury can be reduced or prevented by a diurnal fluctuation in air temperature, as seen in eggplant (Murage et al., 1997), potato (Tibbitts et al., 1990; Cao and Tibbitts, 1991; Cushman and Tibbitts, 1996) and tomato (Hillman, 1956; Ohyama et al., 2005; Sysoeva et al., 2012; Shibaeva and Sherudilo, 2015). Therefore, we carried out the preliminary experiment to investigate the effects of 5 and 10°C of air temperature fluctuation on suppression of leaf browning. As the result, the degree of leaf browning was suppressed significantly by fluctuating air temperature fluctuation of 28°C with 5 and 10°C difference compared to constant 28°C, although suppressive effect was not perfect.

In experiment 1 of the present study, consistent with the preliminary experiment mentioned above, leaf browning of sesame seedlings under continuous lighting was prevented by diurnal fluctuation of air temperature, especially with a 15°C temperature difference (Figs 1 & 2). However, in experiment 2, leaf browning of sesame seedlings also occurred under 12-h photoperiod at constant temperature (Fig. 3), and results of the statistical analysis showed that the only factor to consistently affect the occurrence of leaf injury was temperature (Table 2). The combined results of both experiments therefore suggest that leaf injury in sesame seedlings is induced by constant temperature rather than continuous lighting. These results are very important information to obtain healthy growth of sesame seedlings and lead to establishing the technique of producing sesame seedlings with high content of sesamin. And it is also beneficial that the shoot fresh weight of sesame seedlings cultured under 15°C difference and continuous lighting was highest among all the treatments, and the number of nodes was not affected (Table 3).
investigate the effects of the extent of air temperature fluctuation under various average air temper-atures on the occurrence and degree of leaf browning of sesame seedlings in future. Additionally, in the report of Hata et al. (2012), it is seemed that less intensive leaf browning of sesame plants cultivated at constant 28°C in plant factory with pot filled with commercial soil-mix occurred than those cultivated hydroponically at constant 28°C in this report. Thus, using substrate might be affected to the degree of leaf browning of sesame plants induced by cultivation at constant air temperature in plant factory. On the other hands, since it was not mentioned at all that leaf injury of sesame seedlings, cv ‘Kenana’, cultivated with the substrate of sand at day/night root temperature of 25/25°C and air temperature of 30°C constant occurred (Ali et al., 2000), difference of sensitivity to constant air temperature among cultivars might be existed. So the effects of those factors, including substrate and difference among cultivars, on the occurrence and degree of leaf browning induced by constant air temperature in sesame seedlings cultivated hydroponically in plant factory with artificial light should be also investigated in future.

The physiological mechanism underlying leaf browning in sesame plants induced by constant air temperature is unclear. Some studies investigating physiological changes of leaves exposed to continuous lighting have been reported. In tomato plants exposed to continuous lighting, carbohydrates such as sugar and starch were accumulated in the leaves (Bradley and Janes, 1985), and chlorophyll fluorescence (Fv/Fm) in the leaves was decreased (Shibaeva and Sherudilo, 2015). Furthermore, eggplants showed evidence of photo-oxidative damage such as an increase in superoxide dismutase, peroxidase and catalase activities (Murage and Masuda, 1997). On the other hands, Matsuda et al. (2014) investigated the effects of continuous lighting and diurnal temperature difference on the leaf photosynthesis, plant growth development and carbohydrate accumulation in tomato plants. They suggested that continuous lighting and zero of diurnal temperature difference (DIF) independently affected different processes of photosynthetic metabolism in tomato leaf, because continuous lighting reduced the light limited photosynthesis while zero DIF reduced the light saturated photosynthesis, although net photosynthetic rate were lowered by both of continuous lighting and zero DIF. Thus constant air temperature itself seems to have negative effects on the metabolism in leaves, including photosynthesis. Constant temperature and continuous lighting are different controlling environmental factors, whereas they are similar in that they can be used as treatments to eliminate the circadian rhythm of plants. Thus, the parameters controlling leaf injury induced by continuous lighting should first be investigated in any future research on the physiological causes of leaf injury induced by constant temperature in sesame.

For the production of sesamin-rich sesame seedlings as a new functional leaf vegetable, cultivation under continuous lighting is necessary, since it is reported that this increases sesamin content in sesame leaves (Hata et al., 2012). The knowledge obtained in present study, that leaf browning can be prevented by air temperature fluctuation, might be highly beneficial for stable production of sesamin-rich sesame seedlings under continuous lighting in a plant factory. This would also provide a basis for producing sesame seedlings with yet further improvements in functionality, for example, with higher antioxidant activity, higher nutritional composition, etc. Then the effects of diurnal air temperature fluctuation on sesamin content in sesame leaves under continuous lighting in plant factory are unclear and should be investigated.

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