Effects of Air Temperature Regimes on Physiological Disorders and Floral Development of Tomato Seedlings Grown under Continuous Light

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Abstract. Providing continuous light (24-h photoperiod) at a relatively low photosynthetic photon flux (PPF) is one possible way to reduce both initial and operational costs for lighting and cooling during transplant production with an artificial light. However, physiological disorders (i.e., chlorosis and necrosis) are often observed in several species under continuous light with a constant temperature. The objective of this study was to find an effective air-temperature regime under the continuous light to avoid such physiological disorders, and simultaneously enhance floral development, using tomato [*Lycopersicon esculentum* Mill.] as a model. The seedlings with fully expanded cotyledons were grown for 15 d at a PPF of 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, a relative humidity of 70%, and a CO_2 concentration of about 380 $\mu\text{mol}\cdot\text{mol}^{-1}$ (atmospheric standard). Leaf chlorosis was observed when the air temperature was constant regardless of average air temperature (16, 22, or 28 °C). Neither leaf chlorosis nor necrosis was observed when the air temperatures were alternated [periods of high (28 °C) and low (16 °C) air temperatures of 16/8, 12/12, and 8/16 h·d⁻¹]. Faster floral development was observed in the seedlings grown at lower average air temperatures. These results indicated that physiological disorders of tomato seedlings grown under continuous light could be avoided, and at the same time floral development could be enhanced, by lowering the average air temperature through modification of the periods of high and low air temperatures.

The feasibility of a closed system with an artificial light for transplant production has been previously investigated (Kozai et al., 1999). In the closed system, environmental factors (e.g., air temperature, photoperiod, etc.) can be controlled as desired independently of outside weather, and thereby, conditions that are difficult to obtain in a conventional greenhouse, such as a 24-h photoperiod (or continuous light), can be used. These effects on the growth and development of transplants may potentially reduce the production cost or increase the production efficiency in the closed system, and thus, need to be evaluated.

Continuous light with a relatively low photosynthetic photon flux (PPF) is one possible way to reduce the initial and operational costs for lighting and cooling in the closed system (Ohyama and Kozai, 1998). Decreasing the number of lamps will lower the PPF, however,

extending the photoperiod can compensate for a low PPF (Kitaya et al., 1998a). Use of continuous light also has an advantage in that the lifetime of the lamps can be extended, resulting in lower requirements of lamp fixtures (Koontz and Prince, 1986). Lowering the PPF also reduces the heat load (heat energy generation rate) inside the system, resulting in a reduction of the number and/or capacity of air conditioners or heat pumps.

Under continuous light with a constant air temperature, physiological disorders, characterized by leaf chlorosis and necrosis, are often observed in several species, including tomato (Arthur et al., 1930), chrysanthemum (Warrington and Norton, 1991), eggplant (Murage, 1996), potato (Tibbitts, et al., 1990; Cao and Tibbitts, 1992), radish (Warrington and Norton, 1991), and sweet pepper (Nilwik, 1981), etc. These physiological disorders were observed at any constant air temperature (Hillman,

1956). Low air temperature exposure (Withrow and Withrow, 1949) and/or daily temperature alternation (Hillman, 1956) were effective for preventing or moderating these physiological disorders. In addition, exposure to low air temperature during early stage of emergence (about 8 to 12 d after cotyledon expansion) was also effective for enhancing floral development (Calvert, 1957; Lewis, 1953; Saito and Ito, 1962; Wittwer and Teubner, 1956).

The objective of this study was to find an efficient air-temperature regime under continuous light in the closed system for avoiding physiological disorders, and at the same time for enhancing floral development of the seedlings.

Materials and Methods

Plant material and growth conditions. ‘Momotaro’ tomato seeds were sown in the 128-cell plug trays (Nisshin Nohkoh Sangyo Inc., Gunma, Japan) filled with a commercial substrate [50 vermiculite : 50 peatmoss = (v/v), Yanmar Agricultural Equipment Co., Ltd., Tokyo, Japan]. They were placed in a growth chamber for 6 d at a PPF of 0 or 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (0 to 2 or 3 to 5 d after sowing), an air temperature of 25 °C, and a CO_2 concentration of about 380 $\mu\text{mol}\cdot\text{mol}^{-1}$ (or atmospheric standard), for establishment of the plant material.

The seedlings with fully expanded cotyledons were placed on shelves (30 × 60 cm) in growth chambers (Koitoiron 3HN-35MLA; Koito Industries, Ltd., Yokohama, Japan). They were grown under a 24-h photoperiod at a PPF of 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by high-frequency white fluorescent lamps (FHF16EX-W, Matsushita Electric Industrial Co., Osaka, Japan), a relative humidity of 70%, a CO_2 concentration of about 380 $\mu\text{mol}\cdot\text{mol}^{-1}$, and an air current speed of about 0.5 m·s⁻¹. Subirrigation was applied once every 2 to 3 d with water or a commercial nutrient solution (Otsuka Hydroponics Composition, Otsuka Chemical, Tokyo, Japan) containing major nutrient elements of N, P, K, Ca and Mg at 270, 120, 420, 230, and 60 mg·L⁻¹, respectively, and other minor elements of Fe, Cu, Zn, Mo, and B.

Experiment 1 was designed to investigate the effect of constant air temperature on physiological disorders, growth and development of the seedlings (Table 1). Each of 40 seedlings was subjected to three levels of constant air temperature (16, 22, or 28 °C) for 15 d. Experiment 2 was designed to investigate the effect of

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Table 1. Description of the air temperature treatments in Expt. 1 and Expt. 2.

Treatment	Avg air temp (°C)	Period (h·d ⁻¹)		
		16 °C	22 °C	28 °C
Expt. 1				
C16 ^a	16	24	0	0
C22	22	0	24	0
C28	28	0	0	24
Expt. 2				
A20	20	16	0	8
A22	22	12	0	12
A24	24	8	0	16
C28	28	0	0	24

^aIn treatment codes, C and A on the left represented constant and alternating air temperatures; the numbers on the right represented the average air temperatures.

Fig. 1. Micrographs of tomato apical meristems used as standard observation data. Nondifferentiation stage (a; Stage I), flower primordia initiation stage (b; Stage II), calyx development stage (c; Stage III), and inflorescence formation stage (d; Stage IV) are depicted.

average air temperature with modified periods of high and low air temperatures on the physiological disorders, growth, and development of the seedlings. Each of 128 seedlings was subjected to four levels of average air temperature (20, 22, 24, or 28 °C) for 15 d. The average air temperature was altered by varying the periods of high (28 °C) and low (16 °C) air temperatures, in which air temperatures were selected from the maximum and minimum air temperatures in Expt. 1.

The seedlings after the treatments were transplanted into a plastic pot (diameter, 12 cm; 1 seedling/pot) filled with the commercial substrate after the treatments. They were placed in a plastic greenhouse (35°50'N lat., 139°50'E long.), and were grown for 35 d in Expt. 1 or 48 to 61 d in Expt. 2, respectively, during the period from 15 Jan. to 15 May 2000. Either water or the commercial nutrient solution was applied when necessary.

Measurements. Fresh weight (FW), dry weight (DW_s), leaf area (LA), and stem length (SL) per seedling were measured on day 14 in both Expt. 1 and Expt. 2. To determine the flowering position on the main stem, the number of emerged leaves before the differentiation of the first flower cluster (NL_f) was counted 34 d after transplanting to the greenhouse in Expt. 1. Both NL_f and days to anthesis from transplanting (DA) were recorded in Expt. 2. In Expt. 2, dry weight per plant (DW_p) was measured 24 d after transplanting to the greenhouse. The relative growth rate (RGR) between 0 and 24 d after transplanting was estimated as $RGR = (DW_p - DW_s) / (DW_s \times 25)$. Microscopic observation of the apical meristem using a digital microscope (VH-6300; Keyence Co., Osaka, Japan) was performed in Expt. 2 once every 3 d for the first 24 d after transplanting to determine the floral development stage. The floral development stage was divided into four stages (Stage I to IV), as shown in Fig. 1, based on our preliminary observations. A flat shoot apex with leaf primordia was observed in Stage I. The apex formed two hemispherical domes

or flower primordia in Stage II, when flower differentiation had begun. The calyx of the flower was differentiated in Stage III, and another dome was formed subsequently to former one. Four to five domes were formed in Stage IV, and some of them were fully covered with calyx and then formed inflorescences.

Each treatment was repeated three times. Fifteen to thirty transplants were harvested on day 14 for measurements of FW, DW_s, LA and SL in both Expt. 1 and Expt. 2. Fifteen plants were used to determine NL_f, DW_p, and DA. Nine samples were used to observe the apical meristem in Expt. 2. For the statistical analysis, Tukey's HSD test was applied to compare each treatment when the analysis of variance (ANOVA) indicated treatment significance.

Results and discussion

Experiment 1. Effects of constant air temperature on physiological disorders and the growth and development of the seedlings. In Expt. 1, leaf chlorosis was observed regardless of the constant air temperatures; it was remarkable at an air temperature of 22 °C compared with that at 16 or 28 °C. This finding is consistent with a previous study reported by Hillman (1956). FW, DW_s, LA, and SL decreased as the average air temperature decreased (Table 2). NL_f also decreased as the average air temperature decreased (Table 3), suggesting that floral development was enhanced as the average air temperature decreased.

Experiment 2. Effects of average air temperature with modified periods of high and low air temperatures on physiological disorders and the growth and development of the seedlings. In Expt. 2, no physiological disorders were observed under the alternating air temperatures; while leaf chlorosis was observed under a constant air temperature. LA and SL decreased as the average air temperature decreased, namely under longer exposure to low air temperature (Table 2). FW and DW decreased as the average air temperature decreased (Table 2). However, lower NL_f and more rapid floral development were observed in the seedlings with longer exposure to a low air temperature (Fig. 2). NL_f of the plants originating from the seedlings in

A20 decreased by 2.4 and DA was reduced by 5 d compared with those of plants originating from seedlings in C28 (Table 3). RGR between 0 and 24 d after transplanting increased in the plants originating from the seedlings that were exposed longer to low air temperature, suggesting that seedlings grown under longer exposure to a low air temperature had greater growth potential when they were transplanted.

Tomato transplant production under continuous light in the closed system. Floral development and visual characteristics of the seedlings are the important factors in tomato transplant production from the viewpoint of the transplant quality. In Expt. 1, although floral development was enhanced at the lowest constant air temperature, visual characteristics of the seedlings suggested that constant air temperatures were not suitable for producing transplants under continuous light in the closed system because of the leaf chlorosis. In Expt. 2, more rapid floral development was observed under longer exposure to low air temperature, namely lower average air temperature. A compact shape of the seedling, expressed by smaller LA and shorter SL, without leaf chlorosis was obtained under longest exposure to the low air temperature (A20) among the treatments. These results suggested that possible improvement of transplant quality might be achieved under the continuous light in the closed system by lowering the average air temperature with modification of periods of high and low air temperatures.

FW, DW, and LA in C28 were smaller, but SL was greater in Expt. 2 than in Expt. 1. These growth differences may have been caused by the difference in planting density between Expt. 1 (40/0.18 ≈ 200 seedlings/m²) and Expt. 2 (128/0.18 ≈ 700 seedlings/m²). Under a high planting density or a dense canopy, differences in air temperature, water vapor pressure, and CO₂ concentration between inside and outside the canopy were greater than those in the sparse canopy (Kitaya et al., 1998b), possibly due to the limitation of air movement by leaves inside the canopy. Although using a greater air current speed might have decreased the growth differences between Expt. 1 and Expt. 2 due to the enhancement of mass transfer inside the canopy, further investigation is required to clarify the effects of air current speed on the growth and development of seedlings with a high planting density in the closed system.

NL_f obtained in Expt. 2 was slightly greater than that in our previous report (Ohshima et al.,

Table 2. Growth characteristics of the transplants on day 14.

Treatment	Fresh wt (FW, mg)	Dry wt (DW, mg)	Leaf area (LA, cm ²)	Stem length (SL, cm)
Expt. 1				
C16 ^a	1020 c ^y	93 c	25.1 c	5.3 c
C22	2690 b	205 b	58.6 b	9.3 b
C28	3030 a	234 a	64.3 a	10.7 a
Expt. 2				
A20	1330 d	110 c	33.2 c	7.2 d
A22	1680 c	126 b	38.5 b	9.1 c
A24	1880 b	136 ba	43.1 a	10.2 b
C28	2215 a	155 a	43.6 a	12.5 a

^aIn treatment codes, C and A on the left represented constant and alternating air temperatures; the numbers on the right represented the average air temperatures.

^yMean separation within the columns and experiments by Tukey's HSD test at $P < 0.05$.

Table 3 Growth and development of the seedlings after transplanting to the greenhouse.

Treatment	No. of leaves ^z (NL _F)	Days to anthesis (DA)	Relative growth rate ^y (RGR) (g·g ⁻¹ ·d ⁻¹)
Expt. 1			
C16 ^x	7.4 c ^w	---	---
C22	10.8 b	---	---
C28	12.1 a	---	---
Expt. 2			
A20	9.6 c	48.8 c	0.78 a
A22	10.8 b	51.1 b	0.72 a
A24	11.5 a	53.1 a	0.60 ab
C28	12.0 a	53.9 a	0.44 b

^zThe number of emerged leaves before differentiation of the first flower cluster.

^yRGR was estimated as $RGR = (DW_p - DW_s) / (DW_s \times 25)$ where DW_s and DW_p were dry weights of seedlings at the end of the treatments and 24 d after transplanting.

^xIn treatment codes, C and A on the left represented constant and alternating the air temperatures; the numbers on the right represented the average air temperatures.

^wMean separation with in the columns and experiments by Tukey's HSD test at $P < 0.05$.

Fig. 2. Time courses of the floral development stages of plants originating from seedlings from different periods of high and low air temperatures. The periods of high (28 °C)/low (16 °C) air temperatures were 8/16, 12/12, 16/8, and 24/0 h·d⁻¹ in A20, A22, A24 and C28, respectively. Means $\pm$ SE of three replications are shown. For the floral development stages, see Fig. 1.

2005), suggesting slower floral development. The tomato seedlings were grown under similar environmental conditions, but the CO₂ concentration was about three times higher (1000 $\mu\text{mol}\cdot\text{mol}^{-1}$) in our previous report than in Expt. 2. Carvart and Slack (1975) observed more rapid flowering under the treatment with a higher CO₂ concentration (1000 or 1400 $\mu\text{mol}\cdot\text{mol}^{-1}$) before transplanting, suggesting that the higher CO₂ concentration lowered NL_F. If the CO₂ concentration was three times as great as that in Expt. 2, NL_F might have decreased to the same level as our previous report. In addition, the production cost per seedling was minimally affected by the set point of the CO₂ concentration inside the closed system, because little CO₂ escaped from inside to the outside due to limited ventilation, and the cost of CO₂ gas was significantly less than the electric energy for lamps and cooling (Ohyama and Kozai, 1998).

This study demonstrated that lowering

average air temperature with the modification of periods of high and low air temperatures could prevent physiological disorders of tomato transplants grown under continuous light, while enhancing floral development. These findings contribute to improving quality of transplants and to reducing the production cost per transplant in the closed system, since both initial and operational costs can be reduced by using continuous light in the closed system (Ohyama et al., 2003). We hope that the air-temperature regime described in this report will be adopted for the transplant production of other members of the *Solanaceae* family in the closed system.

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