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Photosynthetic Photon Flux, Photoperiod, and CO_2 Concentration Affect Growth and Morphology of Lettuce Plug Transplants

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***Abstract.* Lettuce (*Lactuca sativa* L. cv. Summer-green) plug transplants were grown for 3 weeks under 16 combinations of four levels (100, 150, 200, and 300 μmol·m^{-2}·s^{-1}) of photosynthetic photon flux (*PPF*), two photoperiods (16 and 24 h), and two levels of CO_2 (400 and 800 μmol·mol^{-1}) in growth chambers maintained at an air temperature of 20 ± 2 °C. As *PPF* increased, dry mass (DM), percent DM, and leaf number increased, while ratio of shoot to root dry mass (S/R), ratio of leaf length to leaf width (LL/LW), specific leaf area, and hypocotyl length decreased. At the same *PPF*, DM was increased by 25% to 100% and 10% to 100% with extended photoperiod and elevated CO_2 concentration, respectively. Dry mass, percent DM, and leaf number increased linearly with daily light integral (DLI, the product of *PPF* and photoperiod), while S/R, specific leaf area, LL/LW and hypocotyl length decreased as DLI increased under each CO_2 concentration. Hypocotyl length was influenced by *PPF* and photoperiod, but not by CO_2 concentration. Leaf morphology, which can be reflected by LL/LW, was substantially influenced by *PPF* at 100 to 200 μmol·m^{-2}·s^{-1}, but not at 200 to 300 μmol·m^{-2}·s^{-1}. At the same DLI, the longer photoperiod promoted growth under the low CO_2 concentration, but not under the high CO_2 concentration. Longer photoperiod and/or higher CO_2 concentration compensated for a low *PPF*.**

Artificial lighting is widely used for plant production under controlled environments (Sase and Ling, 1996), and is used as supplementary lighting in greenhouses when light intensity and duration are inadequate for optimal plant growth and quality (e.g., Gaudreau et al., 1994; Mortensen and Moe, 1983). High light intensity is costly and difficult to obtain using common light sources such as fluorescent lamps. Also, high light intensity often increases leaf temperature and can cause leaf burn. On the other hand, plants grown under low light intensity tend to have characteristics similar to those of shade plants, and are sensitive to intense sunlight when transplanted. Longer photoperiods (Ikeda et al., 1988b) and/or CO_2 enrichment (Mortensen and Moe, 1983) may partly compensate for a low light intensity.

Commercial plug transplant production in greenhouses has increased rapidly (Kozai and Ito, 1993) and is now a standard practice in Europe and North America (Nicola and Cantliffe, 1996). In Japan, demand for lettuce plug transplants has been increasing (Ito, 1992). Since the growth and quality of plug transplants are affected by environmental factors such as light, regulation of transplant growth is easier under artificial light than under natural light. Therefore, identification of the optimal environmental conditions for rapid production of high-quality lettuce plug transplants is essential.

Many reports have shown increased fresh and dry mass of lettuce with extended photoperiods (Ikeda et al., 1988a, 1988b; Koontz and Prince, 1986) and CO_2 enrichment (Ikeda et al., 1988a, 1988b; Knight et al., 1988). Fierro et al. (1994) reported that CO_2 enrichment and supplementary lighting in greenhouses increased shoot and root dry mass of tomato and pepper seedlings.

Instead of photosynthetic photon flux (*PPF*), the daily light integral (DLI, the product of *PPF* and photoperiod) is included in many growth and development models as a light variable (e.g., Bruggink and Heuvelink, 1987; Faust and Heins, 1993). Vlahos et al. (1991) reported that low light intensities at the same DLI increased the growth (plant and leaf dry mass, leaf area, and leaf number) of *Achimenes* more than did high light intensities, and suggested that light-use efficiency is higher at lower light intensities combined with longer daylengths. However, little such information is available for production of lettuce plug transplants under artificial light.

The objective of this study was to investigate the effects of *PPF*, photoperiod, and CO_2 concentration on the growth and quality of lettuce plugs. We attempted to determine: 1) whether similar growth and quality can be obtained under the same DLI; and 2) whether longer photoperiod and/or higher CO_2 concentration can compensate for a low *PPF*.

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Materials and Methods

Three 'Summer-green' lettuce seeds were sown in each cell of 200-cell plug trays (26 × 26 × 43 mm/cell, Kubota Co., Japan) containing soil mix (Yanmar Co., Japan). The trays were then covered with wetted paper towels (Day 0) and placed in a growth chamber at an air temperature of 17 ± 1 °C, vapor pressure deficit (VPD) of 0.40 ± 0.20 kPa and a *PPF* of 190 μmol·m^{-2}·s^{-1} with a photoperiod of 16 h, provided by cool-white fluorescent lamps. The paper towels were removed on Day 2. On Day 3, one uniform seedling per cell was established by thinning. The trays were randomly placed in growth chambers under 16 treatments created by all combinations of four levels of *PPF* (100, 150, 200, and 300 μmol·m^{-2}·s^{-1}), two photoperiods (16 and 24 h), and two concentrations of CO_2 (400 and 800 μmol·mol^{-1}). Six DLIs (5.8, 8.6, 11.5, 13.0, 17.3, and 25.9 mol·m^{-2}·d^{-1}) were then obtained under each CO_2 concentration. The growth chambers were maintained at an air temperature of 20 ± 2 °C and a VPD of 0.44 ± 0.24 kPa. The light source was cool-white fluorescent lamps.

Plants were subirrigated with a 1/4-strength nutrient solution (Otsuka Chemical Co., Japan) containing major nutrient elements of N, P, K, Ca, and Mg at 67, 30, 106, 58, and 15 mg·L^{-1}, respectively, and other minor elements of Fe, Cu, Zn, Mo, Mn, and B at 2-d intervals for 2 weeks. On the 3rd week, the plants were subirrigated daily.

Fresh mass (FM), dry mass (DM), leaf area, and leaf number per plant, leaf length (LL) and leaf width (LW) of the first unfolded true leaf, and hypocotyl length (stem length below cotyledons) of eight transplants were measured on Day 21 for each treatment. The percent DM (DM/FM × 100%), ratio of shoot to root DM (S/R), specific leaf area (ratio of leaf area to leaf DM), and ratio of LL to LW (LL/LW) were then calculated.

We qualitatively define high-quality transplants as sturdy and compact. The higher-quality lettuce plug transplants should have a higher percent DM, and lower S/R, specific leaf area, LL/LW, and hypocotyl length.

The statistical analysis was conducted using Excel Statistics for Windows (ver. 1.0.3) to determine the significance of the effects of *PPF*, photoperiod, and CO_2 concentration on the growth and quality of the transplants. A *t* test was conducted to compare the two treatments with the same DLI under each CO_2 concentration.

Results

The ANOVA for the effects of *PPF*, photoperiod, and CO_2 concentration and their interactions on DM, percent DM, S/R, LL/LW, specific leaf area, leaf area, leaf number, and hypocotyl length of the transplants is summarized in Table 1.

Regardless of photoperiod and CO_2 concentration, DM increased with *PPF* (Fig. 1A). At a given *PPF*, DM was increased by 25% to 100% and 10% to 100% with extended photoperiod and elevated CO_2 concentration, respectively. Percent DM and leaf number increased, while S/R, specific leaf area, LL/LW and hypocotyl length decreased as *PPF* increased (Fig. 1B, G, C, D, E, and F). No substantial differences were observed in LL/LW, and hypocotyl length between 200 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF* except for the treatment of a 24-h photoperiod at the low CO_2 concentration (Fig. 1E and F). In the range of 100 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*, leaf area increased with *PPF* under the 16-h photoperiod and low CO_2 concentration, but decreased with *PPF* at the high CO_2 concentration regardless of photoperiod (Fig. 1H).

Under each CO_2 concentration, DM, percent DM, and leaf number increased linearly as DLI increased (Fig. 2A, B, and G). Logarithmic functions were used to fit S/R, specific leaf area, LL/LW, and hypocotyl length with DLI (Fig. 2C, D, E, and F). Leaf area increased under low CO_2 concentration but decreased linearly under high CO_2 concentration as DLI increased (Fig. 2H). Percent DM was higher, and S/R and specific leaf area were lower, under high than under low CO_2 concentration (Fig. 2B, C, and D).

At 8.6 $mol \cdot m^{-2} \cdot d^{-1}$ DLI and low CO_2 concentration, DM, percent DM, specific leaf area, leaf area, and leaf number were higher, while S/R, LL/LW, and hypocotyl length were lower at lower *PPF* with continuous light than at higher *PPF* combined with shorter photoperiod (Table 2). At this DLI and high CO_2 concentration, however, no significant differences between the two combinations were observed in DM, percent DM, S/R, specific leaf area, and leaf number. When DLI was the same, LL/LW and hypocotyl length were generally low with higher *PPF*, except when DLI was 17.3 $mol \cdot m^{-2} \cdot d^{-1}$ at high CO_2 concentration. Specific leaf area at the same DLI was generally low at low *PPF*.

Discussion

Light intensity is one of the key environmental factors influencing transplant growth and quality. As we qualitatively defined, higher quality lettuce plug transplants should have a higher percent DM and lower S/R, specific leaf area, LL/LW, and hypocotyl length. Under the range of *PPF* used in the present experiment, growth increased and quality improved as *PPF* increased. Leaf morphology, which reflected by LL/LW, was affected by *PPF* only in the range of 100 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Generally, there were no substantial differences in LL/LW and hypocotyl length, which are associated with the morphology and compactness of lettuce plug transplants, between 200 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ *PPF*. Since compact plug transplants are required for easy handling and mechanical transplanting (Hamamoto, 1997), a *PPF* of at least 200

Table 1. A summary of ANOVA for the effects of photosynthetic photon flux (*PPF*), photoperiod (PP), and CO_2 concentration (CO_2) on the dry mass (DM), percent DM, ratio of shoot to root DM (S/R), ratio of leaf length to leaf width (LL/LW), hypocotyl length, specific leaf area, leaf area, and leaf number of lettuce plug transplants on Day 21.

Source	DM	Percent DM	S/R	LL/LW	Hypocotyl length	Specific leaf area	Leaf area	Leaf number
PPF	**	**	**	**	**	**	**	**
Photoperiod	**	**	**	NS	**	**	**	**
CO_2	**	**	**	NS	NS	**	NS	NS
PPF × PP	NS	**	**	NS	**	**	**	NS
PPF × CO_2	NS	**	*	NS	NS	**	**	**
CO_2 × PP	NS	NS	**	NS	NS	NS	**	NS
PPF × CO_2 × PP	NS	NS	**	**	NS	*	*	NS

NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

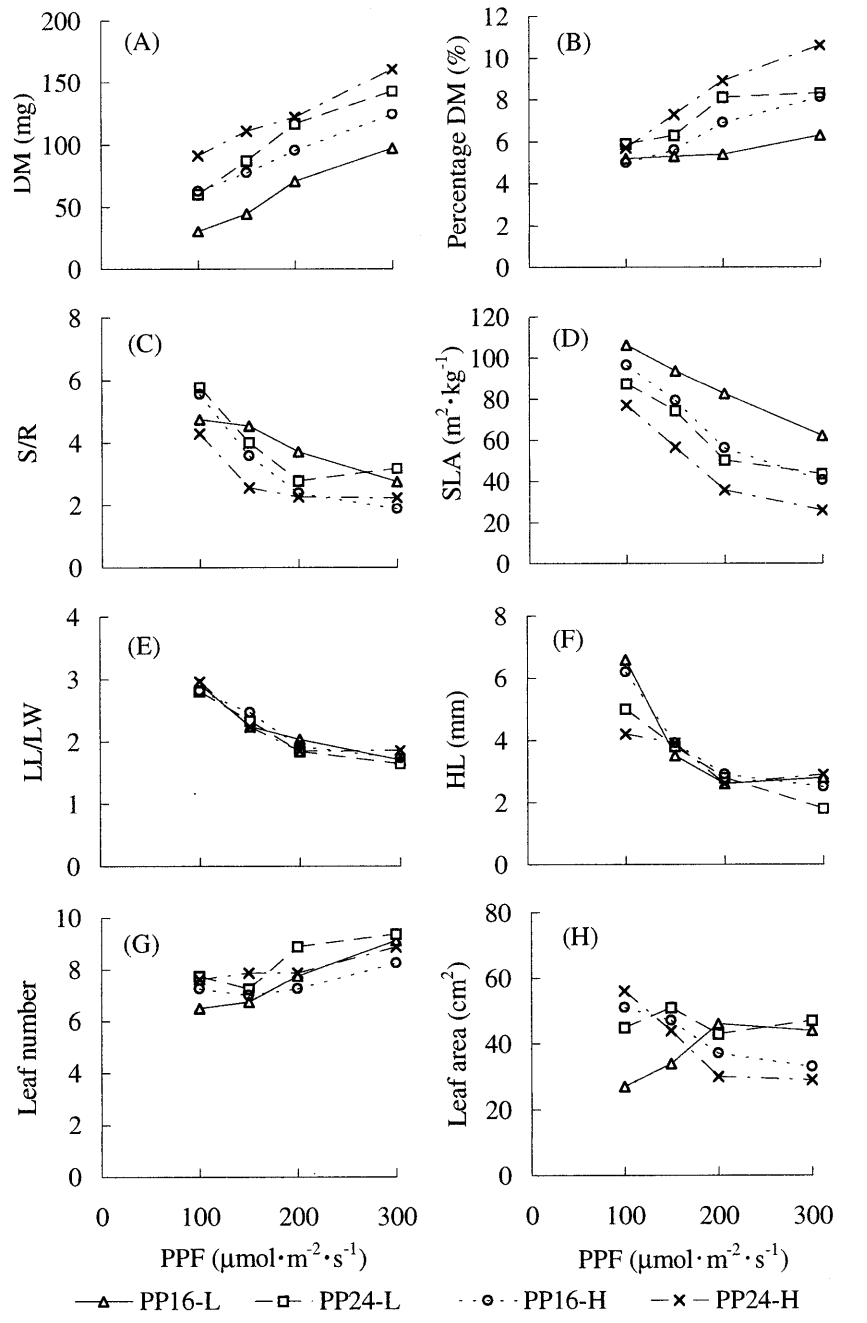

Fig. 1. The effects of photosynthetic photon flux (*PPF*), photoperiod, and CO_2 concentration on (**A**) dry mass (DM), (**B**) percent DM, (**C**) ratio of shoot to root DM (S/R), (**D**) specific leaf area (SLA), (**E**) ratio of leaf length to leaf width (LL/LW), (**F**) hypocotyl length (HL), (**G**) leaf number, and (**H**) leaf area. For treatment symbols, PP16-L (—△—) and PP24-L (– –□–) represent photoperiods of 16 and 24 h, respectively, under low CO_2 concentration; PP16-H (····○····) and PP24-H (–×–) represent photoperiods of 16 and 24 h, respectively, under high CO_2 concentration.

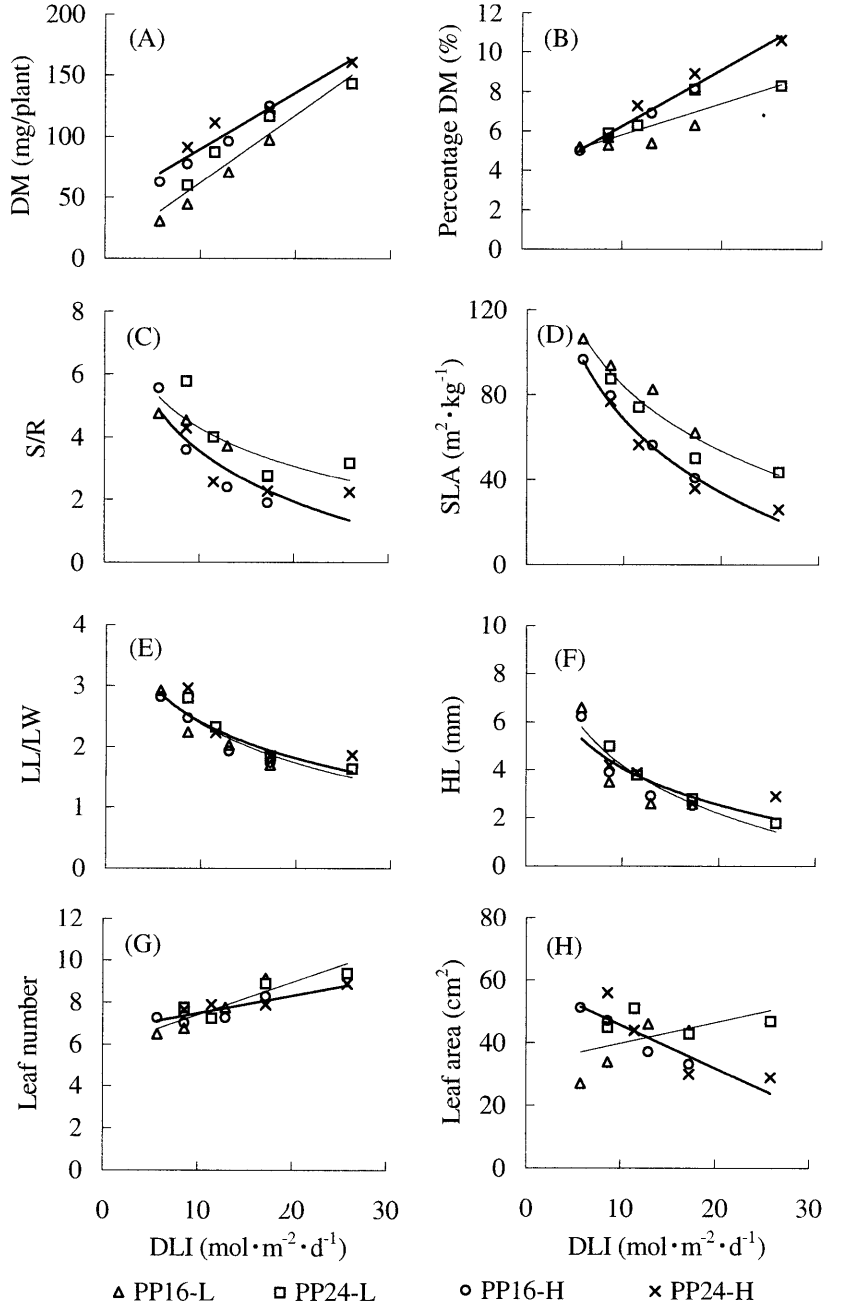

Fig. 2. Relationship between daily light integral (DLI) and (**A**) plant dry mass (DM), (**B**) percent DM, (**C**) ratio of shoot to root DM (S/R), (**D**) specific leaf area (SLA), (**E**) ratio of leaf length to leaf width (LL/LW), (**F**) hypocotyl length (HL), (**G**) leaf number, and (**H**) leaf area. Thick and thin lines represent regression lines under high and low CO_2 concentrations, respectively. Regression equations for high and low CO_2 are: DM = 4.61 × DLI + 43.45 (R^2 = 0.94), and DM = 5.54 × DLI + 6.60 (R^2 = 0.92); percent DM = 0.29 × DLI + 3.40 (R^2 = 0.96), and percent DM = 0.16 × DLI + 4.22 (R^2 = 0.73); S/R = 2.33 × ln (DLI) + 8.94 (R^2 = 0.78), and S/R = 1.77 × ln(DLI) + 8.36 (R^2 = 0.65); SLA = –49.83 × ln(DLI) + 183.23 (R^2 = 0.98), and SLA = –43.59 × ln(DLI) + 184.2 (R^2 = 0.93); LL/LW = –0.85 × ln(DLI) + 4.35 (R^2 = 0.75), and LL/LW = –0.93 × ln(DLI) + 4.51 (R^2 = 0.86); HL = –2.21 × ln(DLI) + 9.17 (R^2 = 0.76), and HL = –2.90 × ln(DLI) + 10.86 (R^2 = 0.82); leaf number = 0.08 × DLI + 6.63 (R^2 = 0.78), and leaf number = 0.15 × DLI + 5.85 (R^2 = 0.83); leaf area = –1.37 × DLI + 59.42 (R^2 = 0.78), and leaf area = 0.66 × DLI + 33.23 (R^2 = 0.30). For treatment symbols, refer to Fig. 1.

μmol·m⁻²·s⁻¹ is recommended for the production of lettuce plug transplants.

Photoperiod can be easily controlled in plant production systems using artificial light. Plug transplants of a number of varieties of floral crops grow more quickly and have higher quality when CO_2 concentration is greater than ambient (Aylsworth, 1996; Porter and Grodzinski, 1985). Longer photoperiod and/or higher CO_2 concentration compensated for a low *PPF*. Dry mass <100 μmol·m⁻²·s⁻¹ *PPF* with 24-h photoperiod and a CO_2 concentration of 800 μmol·mol⁻¹ was similar to that <300 μmol·m⁻²·s⁻¹ *PPF* with a 16-h photoperiod and a CO_2 concentration of 400 μmol·mol⁻¹ (Fig. 1A). Except for leaf number and leaf area, comparable or higher DM, percent DM, and comparable or lower S/R, specific leaf area, LL/LW, and hypocotyl length were observed at 200 μmol·m⁻²·s⁻¹ *PPF* combined with continuous light or high CO_2 concentration than at 300 μmol·m⁻²·s⁻¹ PPF and a 16-h photoperiod (Fig. 1).

Temperature may affect the growth and quality of lettuce plug transplants. Lettuce plants had a lower DM and a higher specific leaf area and S/R at higher than at lower temperatures in a range from 6 to 22 °C under the same light intensities (Lorenz and Wiebe, 1980). Growth and quality of lettuce plug transplants may be influenced by the balance of light and temperature. The ratio of DLI to daily thermal time may be used to quantify the effect of light and temperature (Liu and Heins, 1997). In this experiment, since the temperature was kept constant, the ratio of DLI to daily thermal time is reflected by DLI. We found that growth and quality parameters were generally related to DLI, although modified by CO_2 concentration (Fig. 2). Further research under various temperatures and light levels is required to confirm this observation.

In summary, for a fixed photoperiod and CO_2 concentration, increasing *PPF* promoted the growth and improved the quality of lettuce plug transplants. Extending photoperiod and/or raising CO_2 concentration promoted growth and improved the quality of transplants grown at the same *PPF*. A *PPF* of at least 200 μmol·m⁻²·s⁻¹ is suggested to produce compact lettuce plug transplants under our experimental conditions. Growth and quality of the transplants were related to DLI, although modified by CO_2 concentration. At the same DLI, a longer photoperiod promoted growth under the low CO_2 concentration, while comparable growth was obtained under the high CO_2 concentration. Further experiments are required to verify the results obtained in this study for plants transplanting in greenhouse or field.

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Table 2. Comparison by *t* test of the effects of the two treatments with the same daily light integral (DLI) under two CO_2 concentrations (CO_2) on dry mass (DM), percent DM, ratio of shoot to root (S/R), ratio of leaf length to leaf width (LL/LW), hypocotyl length (HL), specific leaf area (SLA), leaf area, and leaf number of lettuce plug transplants on Day 21.

DLI[z] ($mol \cdot m^{-2} \cdot d^{-1}$)	CO_2 ($\mu mol \cdot mol^{-1}$)	*PPF* ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	PP[y] (h)	DM (mg)	DM (%)	S/R	LL/LW	HL (mm)	SLA ($m^2 \cdot kg^{-1}$)	Leaf area (cm^2)	Leaf number
8.6	400	100	24	60	5.9	5.8	2.8	5.0	87	45	7.8
8.6	400	150	16	45	5.3	4.5	2.2	3.5	94	34	6.8
t test				**	**	**	**	**	**	**	**
17.3	400	200	24	117	8.1	2.8	1.9	2.4	50	43	8.9
17.3	400	300	16	97	6.3	2.7	1.7	2.8	62	44	9.1
t test				**	**	NS	*	NS	**	NS	NS
8.6	800	100	24	91	5.7	4.3	3.0	4.3	77	56	7.6
8.6	800	150	16	77	5.6	3.6	2.5	3.9	79	47	7.0
t test				NS	NS	NS	**	*	NS	*	NS
17.3	800	200	24	122	8.9	2.3	1.8	2.6	36	30	7.9
17.3	800	300	16	124	8.1	1.9	1.7	2.5	41	33	8.3
t test				NS	NS	**	NS	NS	*	NS	NS

[z]DLI = *PPF* × PP × 3600.

[y]PP: Photoperiod.

NS, *, **Nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

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