A Combination of Downward Lighting and Supplemental Upward Lighting Improves Plant Growth in a Closed Plant Factory with Artificial Lighting

Jyotna Joshi
Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Japan; and Mahidol University, Bangkok, Thailand

Geng Zhang
Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Japan; and College of Life Science and Technology, Yangtze Normal University, Chongqing, China

Shanqi Shen
Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Japan

Kanyaratt Supaibulwatana
Mahidol University, Bangkok, Thailand

Chihiro K.A. Watanabe
Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan

Wataru Yamori
Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Japan; and Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan

Additional index words. LED, lettuce, yield, plant factory, photosynthesis, supplemental lighting

Abstract. “Plant factory with artificial lighting” (PFAL) refers to a plant production facility that can achieve mass production of vegetables year round in a controlled environment. However, the high-density planting pattern in PFALs causes low light conditions in the lower canopy, leading to leaf senescence in the outer leaves and thus to reductions in plant yields. In the present study, the effect of supplemental upward lighting underneath the plants on photosynthetic characteristics and plant yield was examined in lettuce, in comparison with supplemental downward lighting from above the plants at the same light intensity. Supplemental upward lighting increased the curvature factor of the photosynthetic response to light from above the plants. Moreover, supplemental upward lighting significantly enhanced the lettuce yield by retarding the senescence of the outer leaves. Here, we propose a novel cultivation system with a combination of downward lighting and supplemental upward lighting that can effectively increase plant growth and yield in PFALs.

In a PFAL, a large number of leafy vegetables (e.g., lettuce and spinach) are grown at a high planting density to achieve high yields. However, this dense cultivation causes a strong shading effect on leaves beneath the plant canopy, leading to a dramatic decrease in photosynthesis in the lower canopy (Brouwer et al., 2012; Twolde et al., 2016). Light is the most important environmental factor affecting photosynthesis and thus yield because plant growth and yield depend on photosynthesis (Yamori, 2016; Yamori and Shikanai, 2016; Yamori et al., 2016). The light intensity in the outer leaves is usually below the photosynthetic light compensation point [i.e., the photosynthetic photon flux density (PPFD) at which photosynthetic rate is zero], meaning that the rate of photosynthesis falls below the rate of respiration (Zhang et al., 2015). The outer leaves senesce fast and appear yellow (McCabe et al., 2001), resulting in large reductions in plant yield at the time of shipment. A recent study indicated that supplemental upward lighting from underneath the plants could delay the senescence of outer leaves (Zhang et al., 2015). However, it is unclear whether supplemental upward lighting from underneath the plants could improve the yield in comparison with supplemental downward lighting from above the plants at the same light intensity because their experiments were examined under different total light intensity. Because the free energy from sunlight is completely excluded from PFALs, additional electrical energy is required to provide growth light for plant cultivation, accounting for as much as 80% of the total electricity consumption (Kozai, 2007; Kozai, 2013a). This causes a great increase in the electricity bill and reduces income substantially. To use the light source efficiently, it is important to investigate the effects of supplemental upward lighting from underneath plant growth in comparison with supplemental downward lighting from above at the same light intensity and propose the lighting environment most suitable to the plant cultivation in PFALs.

Currently, only leafy vegetables and some herbs (shade-type plant species) are suitable for commercial production in PFALs because they can grow well even under relatively low light intensities (100–200 μmol·m²·s⁻¹ PPFD; Kozai et al., 2015). It is difficult to cultivate many vegetables (especially, sun-type plant species), including tomato, paprika, and cucumber, under such low light intensities in a PFAL because they require higher light intensities for growth. The light–response curve of the photosynthetic rate differs between sun- and shade-type plant species. Both the light compensation point and light saturation point of the photosynthetic rate are low in shade species (Givnish, 1988; Lichtenthaler et al., 1981), allowing them to efficiently grow under low light intensities. In contrast, sun species demand more light to drive photosynthesis because they have a higher light compensation point and light saturation point than shade species.
The shape of the photosynthetic light–response curve varies with the direction of irradiation (Moss, 1964; Ögren and Evans, 1993; Terashima, 1986). In general, the photosynthetic light–response curve obtained by irradiating the adaxial side of a dorsiventral leaf shows a higher curvature factor value than that obtained by irradiating the abaxial side of the same leaf (Terashima and Takenaka, 1986). The difference in the curvature factor of the adaxial and abaxial curves became smaller after 7 d of the leaf being inverted and was slightly reversed by 11 d (Terashima, 1986). However, how the photosynthetic light–response curve can be altered by combining downward lighting and supplemental upward lighting has not been examined. If supplemental upward lighting could alter the shape of the photosynthetic light–response curve and thus the light saturation point of photosynthesis, it could make cultivation of sun-type species possible under relatively low downward lighting with supplemental upward lighting. In the present study, to develop a new cultivation system fitting for plant productions under PFALs, we studied the effects of supplemental upward or downward lighting at the same light intensity on photosynthetic characteristics and plant growth in lettuce and measured the light–response curve of photosynthetic rate to analyze whether the supplemental upward lighting could effectively alter the curvature factor of the photosynthetic response to light from above the plants.

Materials and Methods

Plant materials and growth conditions. Romaine lettuce seeds (Lactuca sativa L. var. Romana; Takii Seed Co., Kyoto, Japan) were sown in urethane cubes (W 2.3 cm × D 2.3 cm × H 2.7 cm), and the seedlings were grown in an environmentally controlled growth chamber at 20/17 °C (photoperiod/dark period) under a PPFD of 350 ± 10 μmol·m⁻²·s⁻¹ from cool white fluorescent lamps with a 12-h photoperiod. At 4 weeks after sowing, uniformly sized seedlings at the three-leaf stage were transplanted to a cultivation room under a 25/20 °C photoperiod/dark period temperature (Wang et al., 2016; Zhang et al., 2015) and 14-h photoperiod. The plants were grown in a deep-flow hydroponic system, supplied with Enshi formula nutrient solution (EC: 2.0 ± 0.2 and pH: 7.0 ± 0.5; Asao et al., 2013; Zhang et al., 2015). The cultivation system could provide light both from above (i.e., downward lighting) and underneath (i.e., upward lighting) the plants (Fig. 1). The light treatments were divided into three groups: 1) control: plants were grown solely under downward lighting at a PPFD of 200 μmol·m⁻²·s⁻¹; 2) supplemental downward lighting: plants were grown under downward lighting at a PPFD of 200 μmol·m⁻²·s⁻¹ with supplemental downward lighting at a PPFD of 30 or 60 μmol·m⁻²·s⁻¹; and 3) supplemental upward lighting: plants were grown under downward lighting at a PPFD of 200 μmol·m⁻²·s⁻¹ with supplemental upward lighting at a PPFD of 30 or 60 μmol·m⁻²·s⁻¹. Leaf characteristics. In plants grown without any supplemental lighting, the total chlorophyll content (Fig. 2A) and Fv/Fm (Fig. 2B) remained high in the inner leaves (fourth to sixth layer), but dramatically decreased from the third to first layer in the outer leaves. Similar results were obtained when plants were grown with supplemental downward lighting. Supplemental upward lighting resulted in higher total chlorophyll content and Fv/Fm values in the outer leaves of plants compared with plants grown without any supplemental upward lighting (Fig. 2A and B). In addition, the treatment with supplemental upward lighting at a PPFD of 60 μmol·m⁻²·s⁻¹ resulted in a higher total chlorophyll content.

1) Control

2) Supplemental downward lighting

3) Supplemental upward lighting

Fig. 1. Schematic diagram of the experimental design of the present study. In cultivation beds, a lighting system that could provide light both from above (i.e., downward lighting) and underneath (i.e., upward lighting) the plants was installed. The supplemental light treatments were divided into three groups: (1) control: plants were grown solely under downward lighting at a photosynthetic photon flux density (PPFD) of 200 μmol·m⁻²·s⁻¹; (2) supplemental downward lighting: plants were grown under downward lighting at a PPFD of 30 or 60 μmol·m⁻²·s⁻¹ with supplemental downward lighting at a PPFD of 30 or 60 μmol·m⁻²·s⁻¹; and (3) supplemental upward lighting: plants were grown under downward lighting at a PPFD of 30 or 60 μmol·m⁻²·s⁻¹ with supplemental upward lighting at a PPFD of 30 or 60 μmol·m⁻²·s⁻¹ at the height of the outer leaves. The picture of supplemental upward lighting underneath the plants has been shown.

Results

Leaf characteristics. In plants grown without any supplemental lighting, the total chlorophyll content (Fig. 2A) and Fv/Fm (Fig. 2B) remained high in the inner leaves (fourth to sixth layer), but dramatically decreased from the third to first layer in the outer leaves. Similar results were obtained when plants were grown with supplemental downward lighting. Supplemental upward lighting resulted in higher total chlorophyll content and Fv/Fm values in the outer leaves of plants compared with plants grown without any supplemental upward lighting (Fig. 2A and B). In addition, the treatment with supplemental upward lighting at a PPFD of 60 μmol·m⁻²·s⁻¹ resulted in a higher total chlorophyll content.
Photosynthesis and nitrogen content. In the newest fully expanded leaves (inner leaves; sixth layer), the photosynthetic rate and $g_S$ were highest in the treatment with supplemental downward lighting at a PPFD of 60 $\mu$mol m$^{-2}$ s$^{-1}$, followed by 30 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD, whereas plants in the control and supplemental upward lighting treatments at a PPFD of 60 or 30 $\mu$mol m$^{-2}$ s$^{-1}$ showed similar values (Fig. 3A and B). The nitrogen content in the newest fully expanded leaves (inner leaves; sixth layer) showed a similar trend to the photosynthetic rate in the inner leaves (Fig. 4A).

In the outer leaves (third layer), all the plants without supplemental upward lighting (even those with supplemental downward lighting) showed negative photosynthetic rates (Fig. 3C). However, supplemental upward lighting increased the $g_S$ and photosynthetic rate (Fig. 3C and D). The treatment with supplemental upward lighting at a 60 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD increased the photosynthetic rate in the outer leaves to a greater extent than that at a 30 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD (Fig. 3C). Moreover, supplemental upward lighting increased the leaf nitrogen content in the outer leaves (third layer; Fig. 4B), which showed the same pattern to the photosynthetic rate in the outer leaves (third layer; Fig. 3C).

Photosynthetic light–response curves under different light intensities of supplemental upward lighting. The photosynthetic light–response curves in the newly fully expanded leaves (inner leaves; sixth layer) of control plants were measured to assess the leaf photosynthetic capacity under different levels of supplemental upward lighting (0, 30, 60, or 90 $\mu$mol m$^{-2}$ s$^{-1}$ PPFD; Fig. 5). The maximum photosynthetic rate at the light saturation point was similar in each photosynthetic light–response curve (Fig. 5). However, supplemental upward lighting increased the photosynthetic rate at lower PPFDs compared with the control, whereas the light compensation point was reduced by supplemental upward lighting (Fig. 5). The curvature factor, which indicates the bending rate of the photosynthetic light–response curve, increased with increasing supplemental upward light intensity (Fig. 5; Table 1).

Plant growth. Plant growth and yield were significantly enhanced by both supplemental downward lighting and supplemental upward lighting, but supplemental upward lighting was more effective, and the most remarkable increase was found in the treatment with supplemental upward lighting.
including Rubisco, significantly influences rophyll and photosynthetic enzymes in-rate (Fig. 3) compared with plants grown in marketable leaf fresh weight (a profit of downward lighting (23.3 or 44.8 JPY/electricity, the increased cost of supplement. LEDlighting is a revo-

Discussion

A combination of downward and upward lighting could be an economically feasible and more cost-effective alternative to traditional solely downward lighting. Our results clearly showed that supplemental upward lighting significantly retarded the senescence of outer leaves in plants, resulting in a higher Fv/Fm (Fig. 2B) and higher photosynthetic rate (Fig. 3) compared with plants grown solely under downward lighting. Because nitrogen, an essential constituent of chlorophyll and photosynthetic enzymes in-

Table 1. Parameters for the light–response curve of photosynthetic rate, derived from the nonrectangular hyperbolic response.

| Maximum rate of photosynthesis (Amax) (μmol·m⁻²·s⁻¹) | Curvature factor (θ) |
|-----------------------------------------------------|----------------------|
| 0                                                   | 0.705 ± 0.033 b      |
| 30 up                                               | 14.8 ± 0.7 a         |
| 60 up                                               | 14.6 ± 0.7 a         |
| 90 up                                               | 14.3 ± 0.7 a         |

*“0” denotes the photosynthetic light–response curve measured in leaves without supplemental upward lighting. “30 up,” “60 up,” and “90 up” denote the photosynthetic light–response curve measured in leaves with supplemental upward lighting at 30, 60, and 90 μmol·m⁻²·s⁻¹, respectively. Curves are fitted to the data shown in Fig. 5.*

Fig. 5. Photosynthetic light–response curves of the newest fully expanded leaves (i.e., inner leaves; in the sixth layer) in control plants with different levels of supplemental upward lighting [0, 30, 60, or 90 μmol·m⁻²·s⁻¹ photosynthetic photon flux density (PPFD)]. Data represent means ± SD (n = 5). “200” denotes the photosynthetic light–response curve measured in leaves without supplemental upward lighting. “200 + 30 up,” “200 + 60 up,” and “200 + 90 up” denote the photosynthetic light–response curve measured in leaves with supplemental upward lighting at 30, 60, and 90 μmol·m⁻²·s⁻¹ photosynthetic photon flux density, respectively.

**Fig. 6. (A) Total leaf fresh weights, (A) marketable leaf fresh weights, and (B) wastes of the outer senesced leaves of plants grown under different light treatments at 3 weeks after transplanting. Data represent means ± SD (n = 5). Bars labeled with different letters indicate that the data are significantly different among the five light treatments (Tukey’s HSD test, P < 0.05). Abbreviations are the same as those in Fig. 2.**

834 HORTSCIENCE VOL. 52(6) JUNE 2017
Supplemental upward lighting makes it possible to grow plants with a high light-saturated rate of photosynthesis. At present, the number of plant species suitable for commercial production in PFALs is limited because the light intensity used is relatively low (e.g., below a 200 μmol·m⁻²·s⁻¹ PPFD). Accordingly, only leafy vegetables and some herbs can be produced under such low light conditions (Kozai, 2013b). Low light intensities are insufficient for sun-type plant species, which have a higher light saturation point (Givnish, 1988; Lichtenthaler et al., 1981) and thus need more light for growth. However, our results clearly show that supplemental upward lighting altered the shape of the photosynthetic light–response curve by improving the curvature factor and had a significant effect on the treatment with supplemental upward lighting at the higher light intensity (Figs. 5 and 6; Table 1). This indicates that supplemental upward lighting of the abaxial side of a leaf can lower the saturation point of photosynthesis even under relatively low growth light from above. Our results suggest that a new efficient lighting system with supplemental upward lighting could make it possible to realize PFAL cultivation even for sun-type plant species.

Conclusions

The aim of our research is to establish a new cultivation system in PFALs. The newly proposed system uses a combination of downward lighting and supplemental upward lighting to retard the senescence of the outer leaves and improved the photosynthesis, leading to a significant increase in lettuce yield. Moreover, supplemental upward lighting increased the curvature factor of the photosynthetic light–response curve, providing the feasibility to cultivate sun-type species under relatively low downward lighting with supplemental upward lighting. This novel cultivation system with supplemental upward lighting could be a widespread application in PFALs because it can maximize plant yield through a suitable light source layout and make PFALs applicable to a wide range of plant species.

Asao, T., M. Asaduzzaman, M.F. Mondal, M. Tokura, F. Adachi, M. Ueno, M. Kawaguchig, S. Yanof, and T. Bang. 2013. Impact of reduced potassium nitrate concentrations in nutrient solution on the growth, yield and fruit quality of melon in hydroponics. Sci. Hort. 164:221–231.

Brouwer, B., A. Ziolkoswka, M. Bagard, O. Keech, and P. Gardeström. 2012. The impact of light intensity on shade-induced leaf senescence. Plant Cell Environ. 35:1084–1098.

Givnish, T.J. 1988. Adaptation to sun and shade: A whole-plant perspective. Austral. J. Plant Physiol. 15:63–92.

Kosma, C., V. Triantafyllidis, A. Papasavvas, G. Salahas, and A. Patakas. 2013a. Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. Emir. J. Food Agr. 25:974–979.

Kozai, T. 2007. Propagation, grafting and transplant production in closed systems with artificial lighting for commercialization in Japan. Propag. Ornam. Plants 7:145–149.

Kozai, T. 2013a. Resource use efficiency of closed plant production system with artificial light: Concept, estimation and application to plant factory. Pesc. Jpn. Acad., Ser. B, Phys. Biol. Sci. 89:406–416.

Kozai, T. 2013b. Plant factory in Japan-current situation and perspectives. Chron. Hort. 53:8–11.

Kozai, T., G. Niu, and M. Takagaki. 2015. Plant factory an indoor vertical farming system for efficient quality food production. 1st ed. Massachusetts. Academic press, Cambridge, CA.

Lichtenthaler, H.K., C. Buschmann, M. Dill, H.J. Fietz, T. Bach, U. Kozel, D. Meier, and U. Rahmsdorff. 1981. Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynth. Res. 2:115–141.

McCabe, M.S., L.C. Garratt, F. Scheppers, W.J. Jordi, G.N. Stoopen, E. Davelaar, J.H.A. van Rhijn, J. Brian Power, and M.R. Davey. 2001. Effects of P50G12-IP2 gene expression on development and senescence in transgenic lettuce. Plant Physiol. 127:505–516.

Merrill, B.F., N. Lu, T. Yamaguchi, M. Takagaki, T. Maruo, T. Kozai, et al. 2016. “The next revolution of agriculture: A review of innovations in plant factories”, p. 779–796. In: M. Pessarakli (ed.). Handbook of photosynthesis. 3rd ed, CRC Press, Boca Raton, FL.

Moss, D.N. 1964. Optimum lighting of leaves. Crop Sci. 4:131–136.

Ögren, E. and J.R. Evans. 1993. Photosynthetic light-response curves.I. The influence of CO₂ partial pressure and leaf inversion. Planta 189:182–190.

Porra, R.J., W.A. Thompson, and P.E. Kriedemann. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimi-caet Biophysica Acta (BBA)- Bioenergetics 975:384–394.

Terashima, I. 1986. Dorsiventrality in photosynthetic light response curves of a leaf. J. Expt. Bot. 37:399–405.

Terashima, I. and A. Takenaka. 1986. “Organization of photosynthetic system of dorsiventral leaves as adapted to the irradiation from the adaxial side”, p. 219–230. In: R. Marcell, H. Clijster, and M. Van Pouke (eds.). Biological control of photosynthesis, Springer-Verlag, Berlin, Germany.

Tewolde, F.T., N. Lu, K. Shiina, T. Maruo, M. Takagaki, T. Kozai, and W. Yamori. 2016. Nighttime supplemental LED inter-lighting improves growth and yield of single-Truss tomatoes by enhancing photosynthesis in both winter and summer. Front. Plant Sci. 7:448.

Wang, J., Y. Tong, Q. Yang, and M. Xin. 2016. Performance of introducing outdoor cold air for cooling a plant production system with artificial light. Front. Plant Sci. 7:270.

Yamori, W. 2016. Photosynthetic response to fluctuating environments and photoprotective strategies under abiotic stress. J. Plant Res. 129:379–395.

Yamori, W. and T. Shikami. 2016. Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. Annu. Rev. Plant Biol. 67:81–106.

Yamori, W., E. Kondo, D. Sugira, I. Terashima, Y. Suzuki, and A. Makino. 2016. Enhanced leaf photosynthesis as a target to increase grain yield: Insights from transgenic rice lines with variable Rieske FeS protein content in the cytochrome b₅ complex. Plant Cell Environ. 39:80–87.

Yamori, W., K. Noguchi, K. Hikosaka, and I. Terashima. 2009. Cold-tolerant crop species have greater temperature homeostasis of leaf respiration and photosynthesis than cold-sensitive species. Plant Cell Physiol. 50:203–215.

Yamori, W., K. Noguchi, K. Hikosaka, and I. Terashima. 2010. Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. Plant Physiol. 152:388–399.

Yamori, W., K. Noguchi, and I. Terashima. 2005. Temperature acclimation of photosynthesis in spinach leaves: Analysis of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant Cell Environ. 28:536–547.

Yamori, W., T. Nagai, and A. Makino. 2011. The rate-limiting step for CO₂ assimilation at different temperatures is influenced by the leaf nitrogen content in several C₃ crop species. Plant Cell Environ. 34:764–777.

Yamori, W., G. Zhang, M. Takagaki, and T. Maruo. 2014. Feasibility study of rice growth in plant factories. Rice Res. Open Access 2:139–141.

Yamori, W. and T. Maruo. 2014. Feasibility study of rice growth in plant factories. Rice Res. Open Access 2:119, doi: 10.4172/rjr.1000119.

Zhang, G., S. Shen, M. Takagaki, T. Kozai, and W. Yamori. 2015. Supplemental upward lighting from underneath to obtain higher marketable lettuce (Lactuca sativa) leaf fresh weight by retarding senescence of outer leaves. Front. Plant Sci. 6:1110.

**Fig. 7. Ascorbic acid content in the outer leaves, inner leaves, and total leaves of lettuce plants grown under different light treatments. Data represent means ± SD (n = 5). Bars labeled with different letters indicate that the data are significantly different among the five light treatments (Tukey’s hss test, P < 0.05). Abbreviations are the same as those in Fig. 2.**
Supplemental Fig. 1. The relative spectral photon flux of (A) cool white fluorescent lamps and (B) supplemental downward or upward lighting (white LEDs). The wavelengths of light sources were recorded at 240–800 nm with a spectrometer (SR9910-v7; Irradiant Ltd., Tranent, UK).

Supplemental Table 1. Leaf number and total leaf area of lettuce under different light treatments at harvest time.

| Light treatments | Leaf number | Total leaf area (m²) |
|------------------|-------------|---------------------|
| 200              | 35.2 ± 1.5  | c 0.361 ± 0.288     |
| 200 + 30down     | 40.2 ± 3.0  | c 0.353 ± 0.130     |
| 200 + 60down     | 45.0 ± 1.0  | ab 0.464 ± 0.153    |
| 200 + 30up       | 45.6 ± 1.0  | ab 0.431 ± 0.970    |
| 200 + 60up       | 47.8 ± 1.6  | a 0.469 ± 0.133     |

Data represent means ± SD (n = 5). Values within columns followed by different letters were significantly different (Tukey’s HSD test, P < 0.05).

Supplemental Table 2. Evaluation of the feasibility of different light treatments in real production. Based on local surveys, the retail price of lettuce was 200 JPY/100 g and the electricity bill was 17.49 JPY/KW h. (A) Electricity bill of supplemental upward lighting per plant was calculated as: electricity consumption of white LEDs × 14/1000 × 1/8 × 17.49, where 14 was the photoperiod and 8 was the number of plants illuminated by supplemental white LEDs from underneath the plants; (B) retail price per plant was calculated as: marketable leaf fresh weight/100 × 200; net retail price per plant was calculated as B minus A.

| PPFD (µmol·m⁻²·s⁻¹) | Marketable leaf fresh weight (g) | Electric consumption of supplemental lighting (KWh plant/d) | Electricity consumption during the treatments | (A) Electricity bill of supplemental lighting (JPY plant) | (B) Retail price (JPY plant) | (B – A) Net retail price (JPY plant) |
|----------------------|---------------------------------|----------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------|------------------------------|--------------------------------------|
| 200                  | 143.7 ± 25.2 c                  | 0.000 ± 0.000 c                                          | 0.00 ± 0.00 d                                 | 287.3 ± 50.5 c                                         | 287.3 ± 50.5 c               | 287.3 ± 50.5 c                       |
| 200 + 30down         | 161.8 ± 11.7 c                  | 0.051 ± 0.002 b                                          | 1.33 ± 0.04 b                                 | 323.6 ± 23.6 c                                         | 300.2 ± 23.7 c               | 287.3 ± 50.5 c                       |
| 200 + 60down         | 182.6 ± 3.8 b                   | 0.098 ± 0.001 a                                          | 2.56 ± 0.03 a                                 | 365.2 ± 7.6 b                                         | 320.4 ± 7.2 c                | 287.3 ± 50.5 c                       |
| 200 + 30up           | 194.5 ± 8.4 b                   | 0.048 ± 0.002 b                                          | 1.24 ± 0.05 b                                 | 388.9 ± 16.7 b                                        | 367.3 ± 16.6 b               | 287.3 ± 50.5 c                       |
| 200 + 60up           | 224.5 ± 8.7 a                   | 0.098 ± 0.002 a                                          | 2.55 ± 0.04 a                                 | 449.0 ± 17.4 a                                        | 404.4 ± 17.6 a               | 287.3 ± 50.5 c                       |

Data represent means ± SD (n = 5). Values within columns followed by different letters were significantly different (Tukey’s HSD test, P < 0.05).