Effects of Local CO₂ Enrichment on Strawberry Cultivation during the Winter Season

Yuta MIYOSHI1,2, Kota HIDAKA3, Takashi OKAYASU4, Daisuke YASUTAKE3 and Masaharu KITANO1

1 Postdoctoral Research Fellow of the Japan Society for the Promotion of Science, Japan
2 Takasaki Advanced Radiation Research Institute, National Institutes for Quantum and Radiological Science and Technology (QST), Takasaki, Gunma 370-1292, Japan
3 NARO Kyushu Okinawa Agricultural Research Center, Karume, Fukuoka 839–8503, Japan
4 Faculty of Agriculture, Kyushu University, Higashi-ku, Fukuoka 812–8581, Japan

(Received June 1, 2017; Accepted August 17, 2017)

In strawberry production, carbon dioxide (CO₂) enrichment is widely used to promote crop photosynthesis and to improve growth and yield of strawberry fruits. However, a standard CO₂ enrichment method has not been established clearly. For sustainable strawberry production with high profitability, it is essential to establish an efficient system for CO₂ enrichment. Therefore, in this study, we newly propose a local CO₂ enrichment system. The system enriches CO₂ only ambient to strawberry crops, not a whole greenhouse, and thereby enables efficient control of CO₂ concentration. We applied this system in a strawberry cultivation greenhouse with ventilating windows and examined the performance of system. During the daytime on clear days with opened greenhouse ventilation windows, the proposed system stabilized the ambient CO₂ concentration to strawberry crops at 800 ppm. Furthermore, when the day after CO₂ enrichment was begun, strawberry crop photosynthesis and photosynthate translocation from source leaves were promoted as the effects of short-term CO₂ enrichment. Nevertheless, the yields and quality of fruits was not affected by the system. It is considered that long-term CO₂ enrichment thickened source leaves and thereby caused down-regulation of photosynthesis. Further consideration will be needed on effect of long-term CO₂ enrichment on physiological function of crops.

Keywords : environmental control, greenhouse, photosynthesis, photosynthate translocation, source-sink balance, yield

INTRODUCTION

Carbon dioxide (CO₂) concentration is one of the most important environmental factors in crop production. Within the range of suitable CO₂ concentration for crops, an increase of CO₂ concentration results in an increase in net crop photosynthesis (Cure and Accock, 1986; Keutgen et al., 1997) and thereby promotes photosynthate translocation, which directly controls the crop yield and quality (Troughton and Currie, 1977; David et al., 2014). Therefore, in greenhouse horticulture, CO₂ enrichment is commonly used to realize high crop productivity (Kawashima et al., 2008).

In strawberry production, CO₂ enrichment has been practiced since the 1970s (Kato et al., 2015), and is used widely today in strawberry-cultivation greenhouses (Wada et al., 2010). In areas of scant solar radiation during the winter season, daytime enrichment of CO₂ increased crop yield by 40% (Kawashima, 1991), while in areas of abundant solar radiation, early morning enrichment of CO₂ increased crop yield by 16% (Shigeno et al., 2001). The effect of CO₂ enrichment on increasing yield is well established; however, a normative CO₂ enrichment strategy has not been determined (Mizukami et al., 2011). Moreover, a previous study revealed the ineffectiveness of industry-standard methods for enriching CO₂ (Miyoshi et al., 2013).

At our experimental site in the northern Kyushu area during the winter strawberry production season, CO₂ was generally enriched in the greenhouse using a fuel-burning CO₂ generator only early in the morning. CO₂ concentrations inside the greenhouse began to increase with the beginning of CO₂ enrichment and reached a maximum of approximately 2,000 ppm. However, beginning greenhouse ventilation to cool the greenhouse rapidly decreases the CO₂ concentration inside the greenhouse. Consequently, the CO₂ concentration decreases to outdoor levels before noon, when photosynthetic photon flux density (PPFD) becomes sufficient for strawberry crop photosynthesis. Thus, greenhouse ventilation renders CO₂ enrichment ineffective with regard to increasing photosynthesis in the strawberry crop.

In this study, we propose a new local CO₂ enrichment system. The system enriches CO₂ in close proximity to the crops and thereby enables efficient control of CO₂ concentration even when the greenhouse ventilation system is active. We applied the system to a strawberry greenhouse with ventilation windows to examine the effect of this local CO₂ enrichment. We also examined the effects of CO₂ enrichment system on physiological functions such as photo-
synthesis and photosynthetic translocation, and on growth and yields.

MATERIALS AND METHODS

System for local CO₂ enrichment

For efficient CO₂ enrichment inside the greenhouse, we propose a new local CO₂ enrichment system utilizing the existing system described in our previous study for local environment control (Miyoshi et al., 2013). Figure 1 shows a schematic of this local CO₂ enrichment system. The system was installed in a strawberry-cultivation greenhouse (11 m long × 5 m wide × 3 m high) located at the Kyushu University, Fukuoka, Japan, and consists of inter-row air ducts (IAD; 0.1 m in diameter, 6 m in length, made of aluminum) that are connected to a blower (35 m³/min). CO₂ is added to the IAD, which are perforated to disperse the CO₂-enriched air in close proximity to the crops. The CO₂ was supplied to the IAD by a gas cylinder of liquefied carbon dioxide, and CO₂ concentrations inside the IAD were maintained at 1,000 ppm. Two treatments, CO₂ enrichment (CO₂ Duct) and natural CO₂ conditions (No Duct), were carried out from January 27, 2015, when the primary axillary inflorescence was flowered, to March 1, 2015, when the first flowering of the primary axillary inflorescence was harvested. CO₂ enrichment was applied from 7:00 to 17:00 under the control of timer switches (TB22101, Panasonic Corporation, Nagano, Japan) containing a non-dispersive infrared (NDIR) CO₂ analyzer and a built-in data logger with 10-min intervals was used to measure CO₂ concentration in each cluster of strawberry crops during each treatment.

Measurement of leaf photosynthesis

To analyze the effect of the local CO₂ enrichment system on leaf photosynthesis, we measured leaf photosynthetic rates during CO₂ Duct and No Duct treatments. The measurement were carried out using a portable photosynthesis and fluorescence system (LI-6400XT, LI-COR Inc., USA) using a chamber head with a natural light window under ambient air temperature, CO₂ concentration, and relative humidity conditions. Six plants were selected from each treatment for photosynthetic measurement, and the photosynthetic rates were measured for the third expanded leaf. Differences between treatments were tested for statistical significance using the Student’s t-test at P < 0.05.

Measurement of sucrose concentrations

To analyze the effect of local CO₂ enrichment system on photosynthetic translocation of strawberry crops, we measured the amount of change in leaf sucrose concentration, which represents the translocated photosynthetic sucrose (Sutsawat et al., 2008), during the daytime from 6:00 to 18:00 and during the nighttime from 18:00 to 6:00 (Miyoshi et al., 2017). We collected samples at the source leaves of the strawberry, sampling from each third expanded leaf of the six plants in each treatment using a 6 mm diameter punch, while avoiding the major vein. We performed quantitative analysis on the collected samples using high-performance liquid chromatography (LC-20A, SHIMADZU CORPORATION, Japan); yielding the sucrose concentration per 1 mL of extracted solution. The molar concentration of sucrose per unit area of the source leaf was calculated as the acquired sucrose.
LOCAL CO₂ ENRICHMENT

concentration value divided by the molar mass of sucrose (342.3 g mol⁻¹) and the area of the collected samples. Differences between treatments were tested for significance using the Tukey-Kramer test at \( P < 0.05 \).

Analysis of growth and yield

To analyze the long-term effect of CO₂ enrichment on strawberry growth and yield, we calculated the leaf area of fully expanded third leaves for six plants under each treatment, using the length \( L_c \) and width \( W_c \) of leaflets and the following equation described previously by Hidaka et al. (2013):

\[
\text{Leaf area} = 1.658 \times (L_c \times W_c) + 2.495
\]

We also calculated growth characteristic parameters including specific leaf weight \( SLW \) (an index of leaf thickness) for six plants under each treatment using the following equation described previously by Hidaka et al. (2015):

\[
SLW = \text{leaf dry weight per plant/leaf area per plant}
\]

Furthermore, to analyze the effect on yield, was harvested from six plants for each treatment. Differences between treatments were tested for significance using the Tukey-Kramer test at \( P < 0.05 \).

RESULTS AND DISCUSSION

Performance and effectiveness of the system

Figure 2 shows diurnal changes in CO₂ concentration within strawberry crop clusters during CO₂ Duct and No Duct treatments during clear days (Feb. 3, 2015) when the CO₂ enrichment was begun before one week. During CO₂ Duct treatment, CO₂ concentration began to increase with the commencement of CO₂ enrichment (7:00), and remained around 800 µmol mol⁻¹ in the proximity of the strawberry crop. At 11:00, when greenhouse ventilation windows were opened, CO₂ concentration decreased rapidly to 600 µmol mol⁻¹, but quickly recovered to 800 µmol mol⁻¹. Then, after 11:00, while ventilation windows remained open, CO₂ concentrations remained around 800 µmol mol⁻¹. After the cessation of CO₂ enrichment (17:00), CO₂ concentrations decreased to 400 µmol mol⁻¹.

During the No Duct treatment, CO₂ concentrations gradually decreased due to crop photosynthesis after sunrise, reaching a minimum of approximately 300 µmol mol⁻¹ at 11:00. After ventilation windows were opened, CO₂ concentrations remained around 400 µmol mol⁻¹. From these results, it is suggested that efficient CO₂ enrichment can be made possible by using local CO₂ enrichment systems in strawberry greenhouses in which window ventilation is necessary for avoiding air temperature increases.

Figure 3 shows photosynthetic rates of fully expanded third leaflets under CO₂ Duct treatment and No Duct treatment at 12:00 on a clear day (Feb. 3, 2015) one week after the beginning of CO₂ enrichment. Photosynthetic photon flux density, air temperature, and relative humidity were relatively similar for both treatments at approximately 600 µmol m⁻² s⁻¹, 28°C, and 60%, respectively. CO₂ concentrations were 800 µmol mol⁻¹ during the CO₂ Duct treatment and 400 µmol mol⁻¹ during the No Duct treatment. Photosynthetic rates were 15 µmol CO₂ m⁻² s⁻¹ during the CO₂ Duct treatment and 10 µmol CO₂ m⁻² s⁻¹ during the No Duct treatment. Photosynthetic rate was
significantly higher during the CO2 Duct treatment, measuring 1.5 times the rate measured during the No Duct treatment. It is suggested that CO2 enrichment accelerated leaf photosynthesis.

Figure 4 shows the daily amount of sucrose loading, which is the photosynthate translocation from leaves to phloem (Sylvie et al., 1999), under CO2 Duct and No Duct treatments on a clear day (Feb. 3, 2015) calculating by the equation described previously (Miyoshi et al., 2017). Daily amounts of sucrose loading were significantly higher during the CO2 Duct treatment, measuring 1.5 times the amounts measured during the No Duct treatment, similar to the differences in photosynthetic rate discussed above. High CO2 concentrations promote higher photosynthetic rate, causing an increase in photosynthate concentration in source leaf (Lemoine et al., 2013), and, in turn, high concentrations of photosynthate promote photosynthate translocation from source leaf (Ainsworth and Bush, 2011). Therefore, it is suggested that CO2 enrichment will promote sucrose loading from the source leaves of strawberry crops.

Figure 5 shows the leaf area of the fully expanded third leaf under CO2 Duct treatment and No Duct treatment on March 1, 2015, one month after the beginning of CO2 enrichment. Leaf area was significantly larger during the CO2 Duct treatment, measuring 1.3 times the rate measured during the No Duct treatment. Figure 6 shows the specific leaf weight (SLW) of the fully expanded third leaf under each treatment on the same day. SLW was significantly greater during the CO2 Duct treatment, measuring 2 times the rate measured during the No Duct treatment. On the other hand, the Brix of harvested fruit of first flower at apical inflorescences showed no significant difference between CO2 Duct and No Duct treatments (Fig. 7). Similarly, yield and number of fruits showed no significant difference between treatments (Fig. 8).

Poorter (1993) reported that plant biomass increases under CO2 enrichment treatment. Lemoine et al. (2013) also reported that an increase in CO2 concentration should enhance photosynthetic rates and carbohydrate production, and have a positive effect on photosynthate transport and growth. In fact, most plants grown in high CO2 environments exhibit increased carbohydrate accumulation in leaves with biomass partitioning between source and sink organs (Makino and Mae, 1999). However, classically, there are two high-CO2 acclimation steps-short-term and long-term acclimation - and physiological functions such as photosynthetic rate and photosynthate translocation are initially enhanced only during short-term exposure; the boost of growth under high CO2 concentrations is not sustained long-term (Drake et al., 1997; Cheng et al., 1998). The short-term response to high CO2 concentration is an acclimation process whereby net photosynthesis, net carbon assimilation, and photosynthate translocation are enhanced. Excess photosynthate in source organs is only partly exported to sink organs via the phloem, and the resulting carbohydrate accumulation in leaves decreases the photosynthesis rate (Baie and Sicher, 2004). Furthermore, Hidaka et al. (2014) reported that long-term exposure of strawberry plants to the optimal environment for leaf photosynthesis induced down-regulation of leaf photosynthesis as plants limited the sink demand. From these previous researches, it is considered that one of the reasons for ineffectiveness of CO2 enrichment on Brix and yields is down-regulation of photosynthesis in source leaf caused by soluble sugar storage which is led to long-term enrichment of CO2.

In this study, we proposed a new local CO2 enrichment...
studies have shown that the down-regulation of leaf photosynthesis, previous efforts to storage of excessive soluble sugar in source leaf, and, therefore, photosynthesis is down-regulated. In an effort to increase fruit yield. For this new local CO2 enrichment system. The system enabled the efficient enrichment of CO2 in proximity to the crops during ventilation of greenhouse. Furthermore, photosynthesis of strawberry crop and sucrose loading were promoted because of high CO2 concentration immediately after the start of CO2 enrichment. However, marketable yield and degree Brix of harvested fruit showed no significant difference between CO2 - enriched and un-enriched conditions. As one of the reasons for ineffectiveness of CO2 enrichment on yiled and Brix, it is possibly considered that long-term CO2 enrichment leads to storage of excessive soluble sugar in source leaf, and, therefore, photosynthesis is down-regulated. In an effort to avoid the down-regulation of photosynthesis, previous studies have shown that the down-regulation of leaf photosynthesis in tomato plants only occurred when the number of sinks was considerably reduced under elevated CO2: treatment (Qian et al., 2012). Furthermore, Hidaka et al. (2016) suggested that adjusting the timing of initiation of CO2 enrichment to correspond with the sink strength of plants can increase fruit yield. For this new local CO2 enrichment system, further studies are necessary to adjust the timing and strength of CO2 enrichment and to promote photosynthetic translocation to sink organs in order to increase fruit yield.

ACKNOWLEDGEMENTS

This study was supported by JSPS KAKENHI Grant Number JP17H03895, JP15J07916 and JP17K09858.

REFERENCES

Ainsworth, A. E., Bush, R. D. 2011. Carbohydrate export from the leaf: A highly regulated process and target to enhance photosynthesis and productivity. Plant Physiol. 155: 64–69.

Bae, H., Sicher, R. 2004. Changes of soluble protein expression and leaf metabolite levels in Arabidopsis thaliana grown in elevated atmospheric carbon dioxide. Field Crop. Res. 90: 61–73.

Cheng, S. H., Moore, B. D., Seemann, J. R. 1998. Effects of short- and long-term elevated CO2 on the expression of Ribulose-1, 5-Bisphosphate carboxylase/oxygenase genes and carbohydrate accumulation in leaves of Arabidopsis thaliana (L.) Heynh. Plant Physiol. 116: 715–723.

Cure, J. D., Acock, B. 1986. Crop responses to carbon dioxide doubling: A literature survey. Agri. For. Meteorol. 38: 127–145.

David, M. B., Lu, W., Yong-Ling, R. 2014. Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signaling to enhance crop yield and food security. J. Exp. Bot. 65: 1713–1735.

Drake, B. G., González-Meler, M. A., Long, S. P. 1997. More efficient plants: A consequence of rising atmospheric CO2? Annu. Rev. Plant Physiol. Plant Mol. Biol. 48: 609–639.

Hidaka, K., Dan, K., Imamura, H., Miyoshi, Y., Takayama, T., Sameshima, K., Kitano, M., Okimura, M. 2013. Effect of supplemental lighting from different light sources on growth and yield of strawberry. Environ, Control Biol. 51: 41–47.

Hidaka, K., Okamoto, A., Araki, T., Miyoshi, Y., Dan, K., Imamura, H., Kitano, M., Sameshima, K., Okimura, M. 2014. Effect of photoperiod of supplemental lighting with light-emitting diodes on growth and yield of strawberry. Environ. Control Biol. 52: 63–71.

Hidaka, K., Dan, K., Imamura, H., Takayama, T., Sameshima, K., Okimura, M. 2015. Variety comparison of effect of supplemental lighting with LED on growth and yield in forcing culture of strawberry. Environ. Control Biol. 53: 135–143.

Hidaka, K., Dan, K., Miyoshi, Y., Imamura, H., Takayama, T., Kitano, M., Sameshima, K., Okimura, M. 2016. Twofold increase in strawberry productivity by integration of environmental control and movable beds in a large-scale greenhouse. Environ. Control Biol. 54: 79–92.

Kato, K., Kobayashi, K., Shimamoto, C., Nakamura, Y., Kojima, H., Oyabu, T., Ban, Y., Iwasaki, Y. 2015. Effect of mist spray and long-time CO2 enrichment on growth and yield of strawberry in forcing culture. Res. Bull. Aichi Agric. Res. Ctr. 47: 51–60.

Kawashima, N. 1991. Studies on the CO2 enrichment in a greenhouse (3) Effect on the growth of strawberry. Bull. Nara Agri. Expt. Sta. 22: 65–72.

Kawashima, H., Takaichi, M., Baba, M., Yasui, K., Nakano, Y. 2008. Effects of energy saving and the reduction of carbon dioxide emissions with a hybrid-heating system using an air-to-air heat pump for greenhouse heating. Bull. Natl. Inst. Veg. Tea Sci. 7: 27–36.

Keutgen, N., Chen, K., Lenz, F. 1997. Responses of strawberry leaf photosynthesis, chlorophyll fluorescence and macronutrient contents to elevated CO2. J. Plant Physiol. 150: 395–400.

Lemoine, R., Camera, L. S., Atanassova, R., Dédaüdéchamp, F., Allario, T., Pourtau, N., Bonnemain, J., Laloï, M., Thévenot, C. P., Mauroisset, L., Faucher, M., Giroussé, C., Lemonnier, P., Parrilla, J., Durand, M. 2013. Source-to-sink transport of sugar and regulation by environmental factors. Front. Plant Sci. 4: 1–21.

Makino, A., Mae, T. 1999. Photosynthesis and plant growth at elevated levels of CO2. Plant Cell Physiol. 40: 999–1006.

Miyoshi, Y., Hidaka, K., Okayasu, T., Hirano, O., Yasutake, D., Kitano, M. 2013. Application of the constant soil
temperature layer for energy-saving control of the local environment of greenhouse crops. I. Local control of the ambient environment of strawberry. Environ. Control Biol. 51: 89–94.

Miyoshi, Y., Hidaka, T., Hidaka, K., Okayasu, T., Yasutake, D., Kitnao, M. 2017. Dynamics of photosynthate loading in strawberries affected by light condition on source leaves. Environ. Control Biol. 55: 53–58.

Mizukami, K., Hirata, Y., Moriyama, T. 2011. Effective CO₂ enrichment regimes for yield increases in high-bench strawberry culture in Northern Kyushu. Bull. Fukuoka Agric. Res. Cent. 30: 34–39.

Poorter, H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. Vegetatio 1: 77–97.

Qian, T., Dieleman, J. A., Elings, A., Marcelis, L. F. M. 2012. Leaf photosynthetic and morphological responses to elevated CO₂ concentration and altered fruit number in the semi-closed greenhouse. Sci. Hortic. 145: 1–9.

Shigeno, T., Tochigi, H., Oohashi, Y., Inaba, Y. 2001. Effect of electric illumination, carbon dioxide supplementation and underground heating on the growth and yield of strawberry “Tochiotome” in forcing culture. Bull. Tochigi Agr. Exp. Stn. 50: 39–49.

Sutsawat, D., Yamada, K., Shiratake, K., Kanayama, Y., Yamaki, S. 2008. Properties of sorbitol dehydrogenase in strawberry fruit and enhancement of the activity by fructose and auxin. J. Jpn. Soc. Hortic. Sci. 77: 318–323.

Sylvie, L., Eckhard, B., Hanjo, H., Laurence, B., John, W. P., Wolf, B. F., John, M. W. 1999. The dual function of sugar carriers: transport and sugar sensing. Plant Cell 11: 707–726.

Troughton, H. J., Currie, B. G. 1977. Relations between light level, sucrrose concentration, and translocation of carbon 11 in Zea mays leaves. Plant Physiol. 59: 808–820.

Wada, Y., Soeno, T., Inaba, Y. 2010. Effects of light and temperature on photosynthetic enhancement by high CO₂ concentration of strawberry cultivar tochiotome leaves under forcing or half-forcing culture. Jpn. J. Crop. Sci. 79: 192–197.