Effects of CO₂ Enrichment on Yield, Photosynthetic Rate, Translocation and Distribution of Photoassimilates in Strawberry ‘Sagahonoka’

Ai Tagawa 1,2,*, Megumi Ehara 1,†, Yuusuke Ito 1, Takuya Araki 3, Yukio Ozaki 4 and Yoshihiro Shishido 5

1 Saga Prefecture Agriculture Research Center, Nanri, Kawasaki, Saga 840-2205, Japan; ehara-megumi@pref.saga.lg.jp (M.E.); Ito-yuusuke@pref.saga.lg.jp (Y.I.)
2 Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Motooka, Nishi-ku, Fukuoka 819-0395, Japan
3 Graduate School of Agriculture, Ehime University, Tarumi, Matsuyama, Matsuyama 790-8566, Japan; araki@agr.ehime-u.ac.jp
4 Faculty of Agriculture, Kyushu University, Motooka Nishi-ku, Fukuoka 819-0395, Japan; y.ozaki.255@m.kyushu-u.ac.jp
5 NARO Institute of Vegetable and Tea Science, Kannondai, Tsukuba, Ibaraki 305-0852, Japan; yosanys@kpd.biglobe.ne.jp
* Correspondence: tagawa-ai@pref.saga.lg.jp; Tel.: +81-0952-45-2143
† Saga Prefecture Agriculture Technology Center, Nanri, Kawasaki, Saga 840-2205, Japan.

Abstract: The method of automatically controlling the CO₂ concentration in a greenhouse depending on ventilation was examined in order to efficiently improve the productivity of strawberries under the weather conditions in the northern part of Kyushu in Japan. The effects of CO₂ enrichment on the yields, fruit Brix, and economic value of the strawberry ‘Sagahonoka’ were investigated. In addition, in order to clarify the physiological response of ‘Sagahonoka’ to the CO₂ concentration, the photosynthetic rate, translocation, and photoassimilate distribution rate were measured. It was found that maintaining the CO₂ concentrations above 800 µmol mol⁻¹ and 400 µmol mol⁻¹ during no ventilation and ventilation, respectively, resulted in 25% increases in marketable fruit yields and a 0.2–1.2% higher fruit Brix compared to control, which was kept in 400 µmol mol⁻¹ CO₂ or above all day regardless of ventilation. Additionally, the economic value of ‘Sagahonoka’ was increased. The photosynthetic rate of ‘Sagahonoka’ increased linearly up to 800 µmol mol⁻¹ CO₂, and high CO₂ concentrations affected the distribution for the primary fruit, the most significant sink. It was clarified that CO₂ enrichment at 800 µmol mol⁻¹ for ‘Sagahonoka’ was effective in increasing the photosynthetic rate and distribution of photoassimilates to fruits, and the yields of strawberries could be increased efficiently by automatically controlling the CO₂ concentration depending on ventilation in a southern region of Japan.

Keywords: CO₂ concentration; ventilation; yield; economic value; photosynthetic rate; ¹³CO₂; translocation; distribution

1. Introduction

Kyushu is a warm climate region located in the southern part of Japan where forcing the cultivation of strawberries is popular. The growing area for strawberries in Kyushu is 1400 ha, and the shipping amount is 48,410 tons [1], accounting for about 30% of the total of both in Japan. However, the amount of sunshine in winter is low in northern Kyushu [2], i.e., the total solar radiation is below 5.0 MJ m⁻², and this may last for many days during the severe cold season from December to February. Low temperatures in the greenhouse caused by such low solar radiation prevent fruits from ripening, resulting in reduced fruit quality and yield [3]. On the other hand, even in the severe cold season, there are often days when the solar radiation exceeds 10.0 MJ m⁻² under sunny conditions. Therefore,
it is necessary to develop efficient cultivation techniques for increasing fruit quality and yields, responding to various weather conditions. The effects of CO\textsubscript{2} enrichment on plants have been studied in several countries including Japan, and their effectiveness has been shown [4–8]. It has been reported that the fruit yields under 750–1000 \( \mu \text{mol mol}^{-1} \) CO\textsubscript{2} treatments were higher than those without treatment for the strawberries ‘Nyohou’ and ‘Toyonoka’ [9]. However, ventilation is often used to control rises in temperature, and CO\textsubscript{2} flows out from the enriched CO\textsubscript{2} greenhouse to outside by ventilation, resulting in higher costs and impact on the environment. For these reasons, CO\textsubscript{2} application at a concentration higher than that outside is not widespread in northern Kyushu. The fact that greenhouses have to be ventilated during the daytime makes it uneconomical to maintain a high CO\textsubscript{2} concentration [10]. Therefore, in this study, we examined the methods for CO\textsubscript{2} enrichment in consideration of the cost and impact on the environment while achieving the aim of improving the productivity of strawberries under the weather conditions in northern Kyushu. Under the condition of the CO\textsubscript{2} concentration was controlled automatically and maintained to be higher than that outside, depending on ventilation, the yield, fruit quality, and profitability of the strawberry ‘Sagahonoka’ were investigated. Furthermore, in order to clarify the physiological response of the strawberry ‘Sagahonoka’ to the CO\textsubscript{2} concentration, the photosynthetic rate and translocation, and distribution of photoassimilates were investigated.

2. Materials and Methods

2.1. Effects of CO\textsubscript{2} Concentration on Yield, Fruit Brix, and Economic Value

The experiments were conducted in 2 compartments of a plastic greenhouse in the Saga Prefecture Agricultural Research Center in Japan from 15 September 2015 to 30 June 2016 (planting in 2015), and from 16 September 2016 to 30 June 2017 (planting in 2016). Strawberries (\textit{Fragaria} \times \textit{ananassa} Duch. cv Sagahonoka) were cultivated using a bench-culture system (Yazaki Kako Corp., Shizuoka, Japan) with 20 cm spacing between plants and two rows. Coir was filled in cultivation beds, and OAT-A solution (OAT Agrio Co., Ltd., Tokyo, Japan) with an electrical conductivity of 0.6–1.05 mS cm\textsuperscript{-1} was supplied at 50 to 420 mL plant\textsuperscript{-1} day\textsuperscript{-1}. The solution was controlled in response to solar radiation by an integrated environment controller (Maximizer; PRIVA., De Lier, Holland, The Netherlands), and supplied every 1.5 to 2.0 MJ m\textsuperscript{-2} during 8:00–14:00 in both years. The ventilation starting temperature was set at 27 °C during 7:00–12:00 and 24 °C during 12:00–17:00. The relative humidity was maintained above 50% by spraying mist (KYZ75A-4IK; H.IKEUCHI & Co., Ltd., Osaka, Japan) after March. The air temperature was kept above 5 °C using a heat pump (NGP1010T-N; Nepon Inc., Tokyo, Japan). After 15:00, when the outside temperature was less than 7 °C, a vinyl curtain was closed. The dark period was interrupted with lighting time at 22:00–2:00 from December to February.

The CO\textsubscript{2} concentration in the greenhouse was controlled and measured by the integrated environment controller. A CO\textsubscript{2} sensor was placed 20 cm above the base of the plants, and CO\textsubscript{2} was applied as liquefied gas using a porous tube (WTR100; Yamato Jitsugyou Corp, Tokyo, Japan) placed at the base of the plants. Two treatments of CO\textsubscript{2} enrichment were carried out: 800 \( \mu \text{mol mol}^{-1} \) treatment where the CO\textsubscript{2} concentration was kept above 800 \( \mu \text{mol mol}^{-1} \) during no ventilation and 400 \( \mu \text{mol mol}^{-1} \) during ventilation (Figure 1), and as 400 \( \mu \text{mol mol}^{-1} \) treatment where the CO\textsubscript{2} concentration was kept above 400 \( \mu \text{mol mol}^{-1} \) all day, as a control. The CO\textsubscript{2} enrichment for the 800 \( \mu \text{mol mol}^{-1} \) treatment was maintained during no ventilation; therefore, the duration of the 800 \( \mu \text{mol mol}^{-1} \) treatment changed according to the ventilation time depending on the outside weather.
The photosynthetic rate was measured in 2017 and 2018 under the same cultivation conditions in 2015 and 2016. The relationship between CO₂ and photosynthetic rate was measured on 13 February 2017 and the relationship between light and photosynthetic rate was measured on 25, 26, and 29 January 2018. That was measured from 9:00 and 14:00, Tokyo, Japan) every two weeks from January to May. The economic value of applying CO₂ was measured on 13 February 2017 and the relationship between light and photosynthetic rate was investigated under the conditions of a photosynthetic photon flux density (PPFD) of 1000 μmol m⁻² s⁻¹, relative humidity of 70%, leaf temperature of 20 °C, and CO₂ concentration of 0 to 2000 μmol mol⁻¹. The relationship between the PPFD and photosynthetic rate was measured in the fully expanded third leaves of 4 plants. The relationship between CO₂ concentration and photosynthetic rate was measured under the conditions of a CO₂ concentration of 0 to 2000 μmol mol⁻¹ during no ventilation and 400 μmol mol⁻¹ during ventilation. Finally, the net profit for CO₂ treatment was calculated by subtracting the shipping cost and CO₂ enrichment cost from the gross profit.

2.2. Effect of CO₂ Concentration on Photosynthetic Rate

The photosynthetic rate was measured in 2017 and 2018 under the same cultivation conditions in 2015 and 2016. The relationship between CO₂ and photosynthetic rate was measured on 13 February 2017 and the relationship between light and photosynthetic rate was measured on 25, 26, and 29 January 2018. That was measured from 9:00 and 14:00, using a portable photosynthetic transpiration-measuring device (LI-6400, LI-COR Corp., Nebraska, NE, USA) under the condition of 400 μmol mol⁻¹ CO₂ in the greenhouse. The rate was measured in the fully expanded third leaves of 4 plants. The relationship between the CO₂ concentration and photosynthetic rate was measured under the conditions of a photosynthetic photon flux density (PPFD) of 1000 μmol m⁻² s⁻¹, relative humidity of 70%, leaf temperature of 20 °C, and CO₂ concentration of 0 to 2000 μmol mol⁻¹. The relationship between the PPFD and photosynthetic rate was investigated under the conditions of a PPFD of 0 to 1500 μmol m⁻² s⁻¹, relative humidity of 70%, leaf temperature of 20 °C, and CO₂ concentrations of 400 and 800 μmol mol⁻¹.
2.3. Effect of CO$_2$ Concentration on Translocation and Distribution Rate of $^{13}$CO$_2$-Photoassimilates

In order to investigate the translocation and distribution of photosynthetic assimilates, we used ‘Sagahonoka’ cultivated in 18 cm polyethylene pots. On 25 September 2020, the pots were filled with Saga Strawberry Soil (red clay ball soil: palm peat: peat moss: pumice: bark compost: charcoal 10:10:25:35:15:5, boron manganese (BM) heavy-burning phosphorus 2 g/L 10:10:25:35:15:5, BM heavy-burning phosphorus 2 g/L) (JA Saga, Saga, Japan) for bench cultivation and ‘Sagahonoka’ was planted, and 3 pieces of IB Kasei (N:P:K = 10:10:10, N-75 per piece) (JCAM AGRI. Co., Ltd., Tokyo, Japan) were applied. After planting, the same amount of IB Kasei was applied every month. These plants were cultivated in the greenhouse before the flowering stage of the primary fruit in secondary inflorescence and moved to the growth chamber (LPH-Osaka, Japan) on 11 December 2020. The treatments of CO$_2$ enrichment were 400 µmol mol$^{-1}$ and 800 µmol mol$^{-1}$ in the light period. The settings in the growth chamber other than the CO$_2$ concentration were the same for both treatments as follows. The light period was 6:00–18:00, and the dark periods were 18:00–22:00 and 2:00–6:00. At 22:00–2:00, the dark period was interrupted with the lighting time according to normal cultivation. The PPFD of the leaf surface in the light period was about 250 µmol m$^{-2}$ s$^{-1}$, assuming the value in the greenhouse of winter, and it was 160 µmol m$^{-2}$ s$^{-1}$ during the dark-period interruption. The temperature was set at 15 °C at 6:00–7:00, 20 °C at 7:00–8:00, 25 °C at 8:00–16:00, 20 °C at 16:00–17:00, 15 °C 17:00–18:00, and 10 °C at 18:00–6:00. The relative humidity was set at 70% all day. These settings were based on the environment inside greenhouses in northern Kyusyu.

$^{13}$CO$_2$ was fed to the plants at 9:00–10:00 when primary fruits in secondary inflorescence were in anthesis, 12 days after flowering (green ripening stage), and 24 days after flowering (white ripening stage). The plant was arranged in a source–sink unit with 7 leaves and 7 fruits, respectively. $^{13}$CO$_2$ was supplied to the 3rd to 7th leaves in the polyethylene bag with a zipper. A centrifuge tube containing 0.5 g of stable-isotope-labeled barium carbonate ($^{13}$C barium) was in a polyethylene bag. Ten milliliters of 10% lactic acid was added to the $^{13}$C barium, $^{13}$CO$_2$ was supplied to the 3rd to 7th leaves, and the polyethylene bag was opened 1 h after the start of $^{13}$CO$_2$ feeding. Twenty-four hours after the start of $^{13}$CO$_2$ feeding, 4 plants for each treatment were separated. The plant parts, i.e., the source leaves (third to seventh leaf), new leaves (first to second leaves), fruits (primary fruit: top fruit; secondary fruits: second and third fruits; tertiary fruit: fourth to seventh fruits), peduncle, crown, and roots, were separated. The plant parts were analyzed using a stable-isotope analyzer (Integra2 CN, Sercon, Cheshire, UK) after being dried and ground. From the $^{13}$C amount of each part according to the analysis, the translocation and distribution rate were calculated using the following formulas (1) and (2) [12] (pp. 3–4):

Translocation rate = ($^{13}$C amount recovered from all plant parts excluding feed leaves/$^{13}$C amountrecovered from all plant) × 100

Distribution rate = ($^{13}$C amount recovered from each part/$^{13}$C amount recovered from all plant excluding feed leaves) × 100

3. Results

3.1. Effect of CO$_2$ Concentration on Yield, Fruit Brix, and Economic Value the Average Daytime

CO$_2$ concentration over the two years was stably approximately 400 µmol mol$^{-1}$ in the 400 µmol mol$^{-1}$ treatment (Figure 2a). On the other hand, in the 800 µmol mol$^{-1}$ treatment, the average daytime CO$_2$ concentration was about 400 µmol mol$^{-1}$ from October to the middle of November, and gradually rose from the end of November, when the ventilation parts began to remain closed during the day, with a decrease in the total solar radiation and outside temperature (Figure 2a,b). Additionally, the average daytime CO$_2$ concentration remained high from December to February, when the ventilation parts remain closed for longer times during the day. In 2015, the average total solar radiation for every 10 days from December to January was 4.9 to 7.9 MJ m$^{-2}$, and the outside temperature remained low. Therefore, the ventilation was closed for a long time, and the CO$_2$ concentration remained high. In 2016, the average total solar radiation for every 10 days from December to January
was 6.6 to 11.0 MJ m\(^{-2}\), which was higher than that in 2015, and the CO\(_2\) concentration remained slightly lower than in 2016 because of the longer ventilation.

![Graph showing CO\(_2\) concentration and solar radiation](image)

Figure 2. The change in average daytime CO\(_2\) concentration (a), total solar radiation, and daily average outside temperature (b). Daytime means sunrise to sunset; 400 μmol mol\(^{-1}\) treatment where minimum CO\(_2\) concentration is kept more than 400 μmol mol\(^{-1}\) in all day and 800 μmol mol\(^{-1}\) treatment where minimum CO\(_2\) concentration is kept more than 800 μmol mol\(^{-1}\) during no ventilation and 400 μmol mol\(^{-1}\) during ventilation.

Figure 3 shows the change in the ventilation rate, CO\(_2\) enrichment status, CO\(_2\) concentration, and air temperature in the greenhouse, and the solar radiation on a typical sunny day and cloudy day in January. In the 400 μmol mol\(^{-1}\) treatment, the CO\(_2\) concentration did not fall below 400 μmol mol\(^{-1}\) and the CO\(_2\) generator hardly operated on both sunny and cloudy days (Figure 3a,d). On the other hand, in the 800 μmol mol\(^{-1}\) treatment, CO\(_2\) was applied only in the morning and evening when the ventilation parts were closed on a sunny day (Figure 3b), so the average daytime CO\(_2\) concentration on this day was 563.9 μmol mol\(^{-1}\). In the cloudy day in the 800 μmol mol\(^{-1}\) treatment, the ventilation part remained closed throughout the day because the air temperature in the greenhouse did not reach the ventilation temperature, and CO\(_2\) was applied at more than 800 μmol mol\(^{-1}\) during the day (Figure 3c,f), so the average daytime CO\(_2\) concentration on this day was 780.0 μmol mol\(^{-1}\). The air temperature in the greenhouse showed similar changes in both the 400 μmol mol\(^{-1}\) and 800 μmol mol\(^{-1}\) treatments on sunny and cloudy days (Figure 3c,f). No significant differences in plant height and leaf length x leaf width were found between the 400 μmol mol\(^{-1}\) and 800 μmol mol\(^{-1}\) treatments at most of the times (Figure 4). Additionally, there was no significant difference in the marketable fruit rate between the 400 μmol mol\(^{-1}\) and 800 μmol mol\(^{-1}\) treatments. On the other hand, the marketable fruit yield throughout the period and number of marketable fruits and average fruit weight in the 800 μmol mol\(^{-1}\) treatment were larger than those in the 400 μmol mol\(^{-1}\) treatment. The total marketable fruit yields in the 800 μmol mol\(^{-1}\) treatment were 20–31% higher than those in the 400 μmol mol\(^{-1}\) treatment in both years (Table 1). Furthermore,
the fruit Brix of the 800 μmol mol\(^{-1}\) treatment was higher than that of the 400 μmol mol\(^{-1}\) treatment from January to March (Figure 5). The amount of CO\(_2\) used was 57.1 kg a\(^{-1}\) in the 400 μmol mol\(^{-1}\) treatment and 288.1 kg a\(^{-1}\) in the 800 μmol mol\(^{-1}\) treatment. The gross profit was JPY 484,783 a\(^{-1}\) in the 400 μmol mol\(^{-1}\) treatment and JPY 610,693 a\(^{-1}\) in the 800 μmol mol\(^{-1}\) treatment, and the difference was JPY 125,910 a\(^{-1}\). On the other hand, the CO\(_2\) enrichment cost was calculated assuming that LPG was used in both treatments and the CO\(_2\) controller depending on the temperature was used in the 800 μmol mol\(^{-1}\) treatment. The difference between the two treatments was JPY 2900 a\(^{-1}\) for the equipment cost and JPY 12,126 a\(^{-1}\) for the fuel cost. It was estimated that the difference between the gross profit and shipping plus CO\(_2\) enrichment costs was about JPY 83,400 a\(^{-1}\) (Table 2). The exchange rate in November 2021 was USD 1 = JPY 115.

![Figure 3.](image)

**Figure 3.** The change in ventilation rate, CO\(_2\) enrichment status, CO\(_2\) concentration, and air temperature in the greenhouse, solar radiation in the sunny day (a–c) and cloudy day (d–f). The sunny day (a–c) is 28 January 2017, total solar radiation was 14.79 MJ m\(^{-2}\). The cloudy day (d–f) is 12 January 2017, total solar radiation was 5.40 MJ m\(^{-2}\). CO\(_2\) enrichment status means ON: 10, OFF: 0. CO\(_2\) concentration was controlled that 400 μmol mol\(^{-1}\) treatment was kept more than 400 μmol mol\(^{-1}\) in all day and 800 μmol mol\(^{-1}\) treatment was kept more than 800 μmol mol\(^{-1}\) and 400 μmol mol\(^{-1}\) during no ventilation and ventilation, respectively.
Figure 3. The change in ventilation rate, CO2 enrichment status, CO2 concentration (a), Plant height, (b): Leaf length × Leaf width). CO2 concentration was controlled that 400 µmol mol⁻¹ treatment was kept more than 400 µmol mol⁻¹ in all day and 800 µmol mol⁻¹ treatment was kept more than 800 µmol mol⁻¹ and 400 µmol mol⁻¹ during no ventilation and ventilation, respectively. * Is significantly different at 5% levels, respectively, by t-test.

Table 1. Effect of different concentrations of CO2 enrichment on number of marketable fruits, average fruit weight, marketable fruits rate, and marketable fruits yield of strawberry ‘Sagahonoka’.

<table>
<thead>
<tr>
<th>Year</th>
<th>CO2 Treatment (µmol mol⁻¹)</th>
<th>Number of Marketable Fruits per Plant</th>
<th>Average Fruit Weight (g)</th>
<th>Marketable Fruit Rate (%)</th>
<th>Marketable Fruits Yield per Plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11-12</td>
</tr>
<tr>
<td>2015</td>
<td>400</td>
<td>38.6</td>
<td>13.4</td>
<td>81.4</td>
<td>74.6</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>48.0</td>
<td>14.4</td>
<td>87.6</td>
<td>96.5</td>
</tr>
<tr>
<td>2016</td>
<td>400</td>
<td>35.4</td>
<td>15.5</td>
<td>78.8</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>41.4</td>
<td>13.9</td>
<td>80.8</td>
<td>67.1</td>
</tr>
</tbody>
</table>

CO2 concentration was controlled that 400 µmol mol⁻¹ treatment was kept more than 400 µmol mol⁻¹ in all day and 800 µmol mol⁻¹ treatment was kept more than 800 µmol mol⁻¹ and 400 µmol mol⁻¹ during no ventilation and ventilation, respectively. * and ** are significantly different at 1% and 5% levels, respectively, by two-way ANOVA, and ns is not significantly different.

Figure 4. Effects of different concentrations of CO2 enrichment on the growth of strawberry ‘Sagahonoka’ (a): Plant height, (b): Leaf length × Leaf width). CO2 concentration was controlled that 400 µmol mol⁻¹ treatment was kept more than 400 µmol mol⁻¹ in all day and 800 µmol mol⁻¹ treatment was kept more than 800 µmol mol⁻¹ and 400 µmol mol⁻¹ during no ventilation and ventilation, respectively. * Is significantly different at 5% levels, respectively, by t-test.

Figure 5. Effects of different concentrations of CO2 enrichment on the fruit Brix of strawberry ‘Sagahonoka’. CO2 concentration was controlled that 400 µmol mol⁻¹ treatment was kept more than 400 µmol mol⁻¹ in all day and 800 µmol mol⁻¹ treatment was kept more than 800 µmol mol⁻¹ and 400 µmol mol⁻¹ during no ventilation and ventilation, respectively. * and ** are significantly different at 5% and 1% levels, respectively, by t-test.
Table 2. Economic value of strawberry ‘Sagahonoka’ cultivated at different CO₂ concentrations.

<table>
<thead>
<tr>
<th>CO₂ Treatment (µmol mol⁻¹)</th>
<th>CO₂ Amount Used ¹ (kg a⁻¹)</th>
<th>Returns ² (Yen a⁻¹)</th>
<th>Shipping Cost ⁴ (Yen a⁻¹)</th>
<th>CO₂ Application Cost (Yen a⁻¹)</th>
<th>Difference of Returns ⁷ (Yen a⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>57.1</td>
<td>484,783</td>
<td>105,683</td>
<td>1500</td>
<td>2997</td>
</tr>
<tr>
<td>800</td>
<td>268.1</td>
<td>610,693</td>
<td>133,131</td>
<td>4400</td>
<td>12,126</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td></td>
<td>27,448</td>
<td>12,126</td>
<td>83,436</td>
</tr>
<tr>
<td>Rate of 800/400(%)</td>
<td></td>
<td>505</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CO₂ concentration was controlled that 400 µmol mol⁻¹ treatment was kept more than 400 µmol mol⁻¹ in all day and 800 µmol mol⁻¹ treatment was kept more than 800 µmol mol⁻¹ and 400 µmol mol⁻¹ during no ventilation and ventilation, respectively. ¹ Estimated as CO₂ enrichment by circulation fan it used 146% CO₂ by local enrichment. ² Estimated yields of marketable fruits and annual average price in 2015 and 2016 of JA Saga. ³ USD 1 = 115 Yen (November 2021). ⁴ Shipping cost is calculated as 253.7 Yen/kg. ⁵ Rental fee of LPG burning CO₂ generator: 800 µmol mol⁻¹ treatment is estimated adding CO₂ controller depending on air temperature (depreciation period is 7 years). ⁶ Estimated using LPG as 350 Yen/m³. ⁷ Difference of Returns = Returns – Shipping Cost – Equipment Cost – LPG fuel Cost.

3.2. Effect of CO₂ Concentration on Photosynthetic Rate

The photosynthetic rate of ‘Sagahonoka’ rapidly increased as the CO₂ concentration increased up to 800 µmol mol⁻¹ and gradually increased thereafter (Figure 6). The photosynthesis rate at 800 µmol mol⁻¹ CO₂ was 1.6 times higher than that at 400 µmol mol⁻¹ CO₂ (Figure 6). The photosynthetic rate rapidly increased as the PPFD increased up to 300 µmol m⁻² s⁻¹ and gradually increased thereafter under both CO₂ concentrations (Figure 7). The photosynthetic rates at 800 µmol mol⁻¹ CO₂ were significantly higher than the values at 400 µmol mol⁻¹ CO₂ under 100–1500 µmol m⁻² s⁻¹ PPFD. The rates at 800 µmol mol⁻¹ CO₂ were 1.37- and 1.53-times higher than those at 400 µmol mol⁻¹ CO₂ under 100 and 1500 µmol m⁻² s⁻¹ PPFD, respectively. A PPFD of 300 µmol m⁻² s⁻¹ is almost equal to the light intensity in the greenhouse on a cloudy day, and a PPFD of 1000 µmol m⁻² s⁻¹ is almost equal to that on a sunny day. The photosynthetic rate at a PPFD of 300 µmol m⁻² s⁻¹ and 800 µmol mol⁻¹ CO₂ was similar to that at a PPFD of 1000 µmol m⁻² s⁻¹ and 400 µmol mol⁻¹ CO₂.

Figure 6. The relation of CO₂ concentration and photosynthetic rate of strawberry ‘Sagahonoka’. The measurement conditions are PPFD 1000 µmol m⁻² s⁻¹, leaf temperature 20 °C, relative humidity 70%.
Figure 6. The relation of CO$_2$ concentration and photosynthetic rate of strawberry ‘Sagahonoka’. The measurement conditions are PPFD 1000 μmol m$^{-2}$s$^{-1}$, leaf temperature 20 °C, relative humidity 70%.

Figure 7. The relationship PPFD and photosynthetic rate of strawberry ‘Sagahonoka’. ** is significantly different at 1% level by t-test. Error bar means standard error ($n = 4$). The measurement conditions are leaf temperature 20 °C, relative humidity 70%.

3.3. Effect of CO$_2$ Concentration on Translocation and Distribution Rate of $^{13}$CO$_2$-Photoassimilates

The dry matter weight of each part was compared under the conditions of 400 and 800 μmol mol$^{-1}$ CO$_2$ in the growth chamber (Table 3). The dry matter weight of the primary fruit at 800 μmol mol$^{-1}$ CO$_2$ was significantly higher than that at 400 μmol mol$^{-1}$ CO$_2$ 24 days after the flowering of secondary inflorescence. In addition, the dry matter weight of the aerial part at 24 days after flowering was higher than that at the flowering time and 12 days after flowering, and the 800 μmol mol$^{-1}$ CO$_2$ treatment resulted in a higher dry weight of the aerial part compared to 400 μmol mol$^{-1}$ CO$_2$ at 24 days after flowering. The translocation rates for $^{13}$CO$_2$-photoassimilates at 24 days after flowering were 20% higher than those at the flowering time and 12 days after flowering, but they were not affected by the CO$_2$ concentration. The rate of distribution to fruits increased as the number of days after flowering increased, and it was about 30% at 12 days after flowering, becoming 90% or more at 24 days after flowering (Figure 8). The rate of the distribution of the primary fruit was higher under 800 μmol mol$^{-1}$ CO$_2$ than 400 μmol mol$^{-1}$ CO$_2$ at 12 and 24 days after flowering.
Table 3. Effects of different concentration of CO₂ enrichment on dry matter weight of each part of strawberry ‘Sagahonoka’.

<table>
<thead>
<tr>
<th>Experimental Plot</th>
<th>CO₂ Concentration (µmol mol⁻¹)</th>
<th>Period</th>
<th>Leaves (3rd-7th Leaf)</th>
<th>New Leaves (First-Second Leaf)</th>
<th>Total Leaves</th>
<th>Crown</th>
<th>Aerial Part</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering time</td>
<td>-</td>
<td></td>
<td>6.90 ± 0.25 a</td>
<td>3.36 ± 0.24 a</td>
<td>10.26 ± 0.19 ab</td>
<td>2.42 ± 0.12</td>
<td>13.48 ± 0.24 c</td>
<td>6.86 ± 0.41 cd</td>
</tr>
<tr>
<td>12days After</td>
<td>400</td>
<td>7.52 ± 0.25 a</td>
<td>2.21 ± 0.23 c</td>
<td>9.73 ± 0.46 ab</td>
<td>2.79 ± 0.17</td>
<td>14.50 ± 0.31 c</td>
<td>8.62 ± 0.53 bc</td>
<td></td>
</tr>
<tr>
<td>flowering</td>
<td>800</td>
<td>7.53 ± 0.17 a</td>
<td>1.96 ± 0.05 c</td>
<td>9.49 ± 0.19 ab</td>
<td>2.63 ± 0.16</td>
<td>14.38 ± 0.20 c</td>
<td>9.74 ± 0.88 ab</td>
<td></td>
</tr>
<tr>
<td>24days After</td>
<td>400</td>
<td>6.60 ± 0.20 a</td>
<td>2.23 ± 0.15 bc</td>
<td>8.83 ± 0.35 b</td>
<td>2.75 ± 0.06</td>
<td>17.47 ± 0.75 b</td>
<td>10.77 ± 0.26 ab</td>
<td></td>
</tr>
<tr>
<td>flowering</td>
<td>800</td>
<td>7.66 ± 0.39 a</td>
<td>2.99 ± 0.15 ab</td>
<td>10.66 ± 0.47 a</td>
<td>2.88 ± 0.13</td>
<td>20.18 ± 0.66 a</td>
<td>11.07 ± 0.64 a</td>
<td></td>
</tr>
</tbody>
</table>

1. CO₂ enrichment was 400 µmol mol⁻¹ and 800 µmol mol⁻¹ during the light period in the growth chamber.
2. Total leaves = Leaves + New leaves.
3. Aerial part = Total leaves + Fruit bunch + Crown.
5. Total = Aerial part + Roots.

Figure 8. Effects of CO₂ concentration on translocation and distribution rates of ¹³C-photoassimilates. CO₂ enrichment were 400 µmol mol⁻¹ and 800 µmol mol⁻¹ during the light period in the growth chamber.

4. Discussion

CO₂ enrichment in the greenhouse is generally conducted by supplying CO₂ to a closed space, and the applied CO₂ only dissipates to the outside during ventilation. The air temperature rises in the greenhouse even in winter in Tokai and the Southwestern warm regions of Japan. Ventilation is required during the daytime there, which limits the times suitable for CO₂ application and often prevents the full effect of CO₂ application. This is one of the reasons that CO₂ enrichment is not widely used in Japan compared to other countries [13]. Although Saga Prefecture is located in the southwestern warm regions of
Japan, the solar radiation is often low in winter, and ventilation is required for a long time when the weather is clear (Figure 2). Therefore, in order to make the CO$_2$ concentration higher than that of the outside air without waste, the method of automatically controlling the CO$_2$ concentration depending on ventilation, that is, a method of increasing the CO$_2$ concentration in the greenhouse only when the ventilation is closed, was examined. In the 2016 planting, the average total solar radiation for every 10 days from December to January was higher than that in 2015, and the CO$_2$ concentration remained slightly lower than that in 2016 because of the longer ventilation (Figure 2).

The CO$_2$ concentration in the greenhouse of the 800 µmol mol$^{-1}$ treatment fluctuated between sunny days (Figure 3b) and cloudy rainy days (Figure 3e), especially in the severe cold season from December to February; the average daytime CO$_2$ concentration for the 800 µmol mol$^{-1}$ treatment remained higher than that for the 400 µmol mol$^{-1}$ treatment (Figure 2). On the other hand, the marketable fruit yield for the 800 µmol mol$^{-1}$ CO$_2$ treatment was 130% higher than that for the 400 µmol mol$^{-1}$ treatment in 2015, but 120% in 2016. As the average CO$_2$ concentration was kept higher in 2015 than in 2016, it was considered that the rate of increase in yield was high in 2015. According to Kawashima [14], high-concentration CO$_2$ enrichment requires the ventilation in the greenhouse to be closed, so it is considered to be suitable for low-temperature and low-solar-radiation areas. Low-solar-radiation areas in winter, such as Saga Prefecture, have been considered to exhibit weather conditions unfavorable for horticulture in the greenhouse, but a short ventilation time and ability to apply high concentrations of CO$_2$ may be advantageous for horticulture in the greenhouse. Therefore, it is inferred that the results of this study may be effectively utilized in areas of low solar radiation in winter.

It was reported that increasing the CO$_2$ concentration from 300 µmol mol$^{-1}$ to 900 µmol mol$^{-1}$ during the vegetative growth period of strawberries resulted in a significant increase in the dry matter weight of the leaves and roots [15]. However, in this study, there were no effects of the CO$_2$ concentration on the plant height or leaf length x leaf width in the third fully expanded leaf (Figure 4). It is reported that about 90% of the photoassimilates were distributed in the berries at the fruit coloring stage [16]. In this study, it was inferred that the application of CO$_2$ during fruit growth accelerated the distribution of photoassimilates induced by CO$_2$ enrichment to the fruits and did not affect the plant height or leaf size. On the other hand, the number and average fruit weight and total yield of marketable fruits were increased significantly by 800 µmol mol$^{-1}$ CO$_2$ enrichment (Table 1). It has been reported that CO$_2$ enrichment for strawberries resulted in an increase in yield depending on increases in the number of fruits and weight of fruit [9,17,18]. The results of the current study are similar to those reports. The CO$_2$ amount used in the 800 µmol mol$^{-1}$ treatment decreased after the April ventilation time increased, but the marketable fruit yield was significantly higher in the 800 µmol mol$^{-1}$ treatment than in the 400 µmol mol$^{-1}$ treatment throughout the cultivation period. It is said that the role of the increase in the yield of strawberries induced by CO$_2$ enrichment is the maintenance of root activity by maintaining the amount of assimilation and preventing the fatigue of plants [9]. It was considered that the effect of CO$_2$ enrichment in the winter continued even after April, when the period and the amount of CO$_2$ used decreased, in this study, too. In the January–March period, the fruit Brix with the 800 µmol mol$^{-1}$ CO$_2$ treatment tended to be higher than that with the 400 µmol mol$^{-1}$ CO$_2$ treatment (Figure 5). It was previously reported that the sugar content of strawberry fruits is increased by CO$_2$ enrichment [17,19], and the results of this study correspond to these reports. It was surmised that high-concentration CO$_2$ enrichment increased the photosynthetic rate and more photoassimilates were produced, so the sugar content of the fruit increased.

The amount of CO$_2$ used in this study was 57.1 kg in the 400 µmol mol$^{-1}$ CO$_2$ treatment, whereas it was 288.1 kg in the 800 µmol mol$^{-1}$ CO$_2$ treatment (Table 2). According to Kawashima [9], 260 to 400 kg 10a$^{-1}$ of CO$_2$ was required when CO$_2$ was applied at 750 µmol mol$^{-1}$ in a small greenhouse with a ridge height of 2.8 m. In this study, the CO$_2$ concentration in the greenhouse was less frequently below 400 µmol mol$^{-1}$ in the 400 µmol
mol$^{-1}$ CO$_2$ treatment, and the amount of CO$_2$ used was low because the greenhouse had a ridge height of 6.0 m. In this greenhouse, the ridge is high and the inside space is large, so it is thought that CO$_2$ easily diffuses and a large amount of CO$_2$ was required for the 800 μmol mol$^{-1}$ CO$_2$ treatment, but this was a small value in Kawashima’s report. The reason was thought to be the method of CO$_2$ enrichment depending on the ventilation. A high concentration of CO$_2$ in the greenhouse flows out when the ventilation opens [20,21], so it is considered that CO$_2$ enrichment depending on ventilation is very efficient. In recent years, a device that can control the CO$_2$ concentration according to ventilation or temperature in the greenhouse has actually started to be introduced [22]. It will be easy to maintain a high concentration of CO$_2$ using such a device. In this study, strawberries were planted in two rows, and CO$_2$ was applied from a tube placed at the base of the plant in the center of the row. The economic value was calculated based on applying CO$_2$ for the whole greenhouse with a circulation fan, which is easy to introduce, but the CO$_2$ consumption is smaller for local enrichment from the base of the plant. Since the plant height of strawberries is short, it is considered that the CO$_2$ concentration near plants can be increased by applying CO$_2$ locally, and CO$_2$ can thus be applied more efficiently.

Next, in order to verify the physiological response of ‘Sagahonoka’ to changes in CO$_2$ concentration, the effect of the CO$_2$ concentration on the photosynthetic rate was measured. The photosynthetic rate increased sharply as the CO$_2$ concentration was increased up to about 800 μmol mol$^{-1}$; the photosynthetic rate at 800 μmol mol$^{-1}$ was 1.6 times that at 400 μmol mol$^{-1}$ (Figure 6). Among Japanese strawberry varieties, ‘Tochiotome’’s photosynthetic rate is reported in detail [23]. In this study, the photosynthetic rate of ‘Sagahonoka’ tended to saturate at 800–1000 μmol mol$^{-1}$ CO$_2$; similar results have been reported for ‘Tochiotome’. From these results, it was considered that the setting of high CO$_2$ concentration treatment to 800 μmol mol$^{-1}$ is adequate for the purpose of this study. The photosynthetic rate at 800 μmol mol$^{-1}$ CO$_2$ was significantly higher than that at 400 μmol mol$^{-1}$ CO$_2$ when the PPFD was 100 μmol m$^{-2}$ s$^{-1}$ or higher (Figure 7), so it was considered that the photosynthetic rate could be increased by increasing the CO$_2$ concentration even at the light intensity equivalent to dark cloudy weather in the winter greenhouse. Based on this, it was considered that 800 μmol mol$^{-1}$ CO$_2$ treatments enhanced yield production because of the increase in the photosynthetic rate.

Furthermore, the effect of the CO$_2$ concentration on the translocation of photosynthetic assimilates was examined. The growth chamber was set at 400 μmol mol$^{-1}$ CO$_2$ and 800 μmol mol$^{-1}$ CO$_2$, in the light period for 24 days from the flowering stage to the white ripening stage for primary fruit in secondary inflorescence. The dry weight of the aerial part and primary fruit were larger 24 days after flowering than 12 days after flowering and significantly larger with 800 μmol mol$^{-1}$ CO$_2$ than 400 μmol mol$^{-1}$ CO$_2$ 24 days later (Table 3). It has been reported that the sink ability of strawberry fruits is maximized around the fruit coloring stage and then decreases toward the fruit ripening stage [24]. In the authors’ previous study using $^{13}$CO$_2$, the distribution rate for primary fruit at the white ripening stage was the highest among the organs, and it was the largest sink [3,25]. The results of this study are similar to these, and the distribution rate for primary fruit was around 10% 12 days after flowering, but it was the largest, accounting for 50% or more, at 24 days after flowering (Figure 8). However, the distribution rate for primary fruit at 800 μmol mol$^{-1}$ was 121% and 115% of that at 400 μmol mol$^{-1}$ when 12 days and 24th after flowering, respectively. From this, it was clarified that the distribution to the largest fruit had started at 12 days after flowering (green ripening stage), which was considered to contribute to later increasing the dry weight of primary fruit. On the other hand, the photosynthetic rate was higher at 800 μmol mol$^{-1}$ CO$_2$ than 400 μmol mol$^{-1}$ CO$_2$ (Figure 6). Miyoshi et al. [26] reported that the day after CO$_2$ enrichment was started, the strawberry crop photosynthesis and photosynthetic-assimilate translocation from source leaves were promoted; the results in this study indicate the same. Based on this, it was thought that more photosynthetic products were produced at 800 μmol mol$^{-1}$ CO$_2$ than 400 μmol mol$^{-1}$ CO$_2$, and as a result of the large amount of photosynthetic assimilates
being translocated to primary fruit, which is the largest sink, primary fruit 24 days after flowering was significantly larger at 800 µmol mol⁻¹ CO₂ than 400 µmol mol⁻¹ CO₂. Experiments using ¹¹C⁰₂ with a plant positron imaging device (PETIS) revealed that it took about 60 min for the first ¹³C-photoassimilates to reach the fruit in eggplant [27] and in strawberries [28]. Miyoshi et al. [29] reported that the distribution pattern of ¹¹C-photoassimilates translocated to fruits did not change during the light period, nor did the order of the sink activity change. In this study, it was clarified, similarly, that the translocation rate when the same amount of ¹³CO₂ was taken in was not affected by the CO₂ concentration. On the other hand, it is considered that a high CO₂ concentration increased the photosynthetic rate, and many photoassimilates were translocated to the maximum sink; as a result, the size of the sink changed, which affected the distribution rate. In the future, it is expected that the translocation and distribution, in consideration of the sink–source balance at various growth stages, will be further analyzed; this will help in better clarifying the method of obtaining the maximum yield efficiently.

5. Conclusions

It was found that 800 µmol mol⁻¹ treatment that maintained 800 µmol mol⁻¹ CO₂ and 400 µmol mol⁻¹ CO₂ or above during no ventilation and ventilation, respectively, resulted in the marketable fruit yield being increased by 25% and the fruit Brix being 0.2–1.2% higher, compared to 400 µmol mol⁻¹ treatment that maintained 400 µmol mol⁻¹ CO₂ or above all day. Additionally, the economic value of the strawberries increased. Furthermore, the photosynthetic rate of ‘Sagahonoka’ increased linearly up to 800 µmol mol⁻¹ CO₂, and a high CO₂ concentration did not affect the translocation rate but affected the distribution of the primary fruit, which was the greatest sink. It was clarified that CO₂ enrichment at 800 µmol mol⁻¹ for ‘Sagahonoka’ was effective in increasing the photosynthetic rate and the distribution of photoassimilates to fruits, and the yields of strawberries could be increased efficiently by the method of automatically controlling the CO₂ concentration depending on the ventilation in the southern region of Japan.

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